

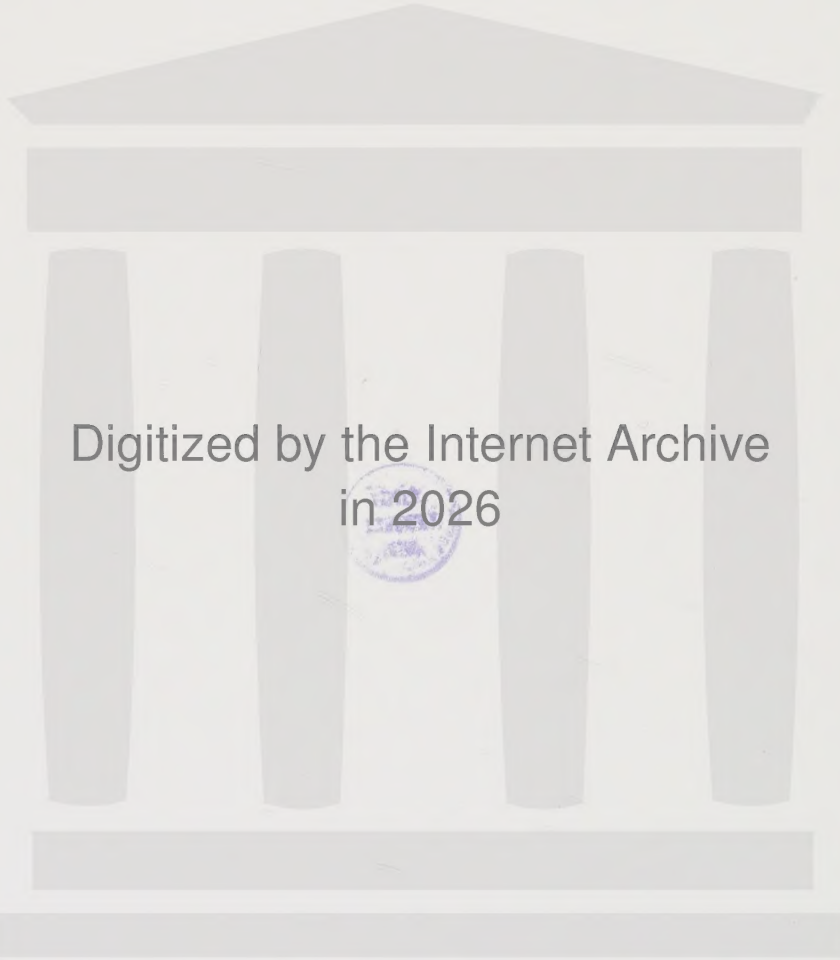
Peter Jerntorp

CHLORPROPAMIDE ALCOHOL FLUSH
AND
ALDEHYDE DEHYDROGENASE ACTIVITY
IN RELATION TO
LATE COMPLICATIONS IN DIABETES MELLITUS



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Title and subtitle Chlorpropamide alcohol flush and aldehyde dehydrogenase activity in relation to late complications in diabetes mellitus			
Abstract The chlorpropamide alcohol flush (CPAF) has been suggested to be of genetic origin and, in type 2 (non-insulin-dependent) diabetics, to be associated with diminished proneness to develop late vascular complications. Objective assessment of the flush, its mechanisms and its relationship to late diabetic complications were studied in 189 type 2 diabetic patients. The objective study of CPAF was found possible, using plasma acetaldehyde measurement and facial temperature methods, an agreement of almost 100% being achieved between the results of these methods used in combination and observer assessment. Some CPAF positive subjects also experienced alcohol flushing without chlorpropamide (CP) premedication. Based on their acetaldehyde concentrations, flushers could be divided into true CPAF positive and false CPAF positive subjects with alcohol flushing. The overall prevalence of true CPAF was 47% in type 2 diabetics, as compared with 14% in healthy male controls. CPAF was prevailingly found in females. CPAF was not associated with diabetes in the family. Plasma CP concentrations were higher in flushers than in non-flushers. CPAF was significantly more common among subjects on regular CP therapy (high CP concentrations) than in those exposed to a single-dose of CP (low CP concentrations). Plasma CP concentrations correlated to the increase in acetaldehyde concentrations after alcohol challenge. By inhibiting aldehyde dehydrogenase (AldDH), CP induced an inhibition of alcohol metabolism. The subsequent increase in the acetyldehyde concentration correlated to individual intensity of facial flushing. Basal AldDH activity in erythrocyte homogenates of subjects unexposed to CP was lower in flushers than in non-flushers. AldDH activity was reduced after CP exposure. CPAF and basal AldDH activity were associated with the occurrence of diabetic complications: AldDH activity was significantly higher in diabetics with late complications than in diabetics free from clinical vascular disease or in healthy controls.			
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Date November 23, 1984

CHLORPROPAMIDE ALCOHOL FLUSH AND ALDEHYDE DEHYDROGENASE ACTIVITY
IN RELATION TO LATE COMPLICATIONS IN DIABETES MELLITUS

Peter Jerntorp

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1985

"NEVER LET GO"

Words of guidance stated by professor Bertil Hood introducing me to the field of internal medicine.

to
Ingela, Sofia and Johan

This thesis is based on the following papers referred to in the text by Roman numerals I-VII:

- I. Jerntorp P, Öhlin H, Bergström B, Almér L-O. Increase of plasma acetaldehyde; an objective indicator of the chlorpropamide alcohol flush. *Diabetes* 1981;30:788-91.
- II. Jerntorp P. The chlorpropamide alcohol flush test in diabetes mellitus: methods for objective evaluation. *Scand J Clin Lab Invest* 1983;43:249-54.
- III. Jerntorp P. Facial skin temperature in chlorpropamide alcohol flush. *Acta Med Scand* 1985; in press.
- IV. Jerntorp P, Almér L-O, Öhlin H, Wåhlin-Boll E, Melander A. Plasma chlorpropamide: a critical factor in chlorpropamide-alcohol flush. *Eur J Clin Pharmacol* 1983;24:237-42.
- V. Jerntorp P, Almér L-O. On plasma chlorpropamide, body weight and sex difference in chlorpropamide alcohol flush (CPAF). *Diabetes Res* 1984;1:223-226.
- VI. Öhlin H, Jerntorp P, Bergström B, Almér L-O. Chlorpropamide-alcohol flushing, aldehyde dehydrogenase activity, and diabetic complications. *Br Med J* 1982;285:838-40.
- VII. Jerntorp P, Öhlin H, Almér L-O. Aldehyde dehydrogenase activity correlates to late diabetic complications. Submitted.

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CONTENTS

ABBREVIATIONS	6
AIMS	7
BACKGROUND	8
MATERIAL AND METHODS	13
RESULTS AND DISCUSSION	18
Methodological studies	18
Pharmacological studies	26
Biochemical studies	30
Clinical studies	39
GENERAL DISCUSSION	43
SUMMARY AND CONCLUSIONS	48
ACKNOWLEDGEMENTS	50
REFERENCES	51
ARTICLES I - VII	63

ABBREVIATIONS

AlDH	Aldehyde dehydrogenase
CBSA	p-Chlorobenzensulfonamide
CBSU	p-Chlorobenzensulfonylurea
CP	Chlorpropamide
CPAF	Chlorpropamide alcohol flush
Flusher	CPAF positive subject
FVD	Free from clinical vascular disease
LVD	Large-vessel disease
MODY	Maturity onset diabetes of the young
Mm-MAST	Malmö modification of brief Michigan Alcoholism Screening Test
Δ MTCI	Change in malar thermal circulation index
NAD^+	Nicotinamide-adenine dinucleotide
NIDD	Non-insulin-dependent, maturity onset, type 2 diabetes mellitus
Non-flusher	CPAF negative subject
Δ P-acetaldehyde	Plasma acetaldehyde increase
ΔT	Facial skin temperature increase
%T	Per cent of maximum possible skin temperature increase
t_0	Basal facial skin temperature
$T_{1/2}$	Individual half-life

AIMS

The principal aims of the studies were:

- to evaluate whether the chlorpropamide alcohol flush (CPAF) could be studied objectively
(Methodological studies),
- to study the biochemical basis of the CPAF reaction
(Pharmacological and Biochemical studies),
- to investigate whether any relationship exists between CPAF, aldehyde dehydrogenase (AldDH) activity and late diabetic complications
(Clinical studies).

BACKGROUND

With the introduction of insulin more than 60 years ago, and peroral anti-diabetic drugs 30 years ago, it became possible to reduce blood glucose concentrations to normal or near normal levels in diabetic patients. The major problem in the treatment of diabetes today is the increasing prevalence of late diabetic complications, such as retinopathy, nephropathy, neuropathy and large-vessel disease (1). In addition to blood glucose imbalance, other factors are likely to be involved in the pathogenesis of these complications (2). This thesis deals with one such factor, a flush phenomenon, possibly related biochemically to late complications in diabetes mellitus.

During recent years it has become clear that the importance of flushing reactions goes beyond their cosmetic consequences. Current interest in flush mechanisms is largely the result of speculations that one of the flush reactions may identify a population at risk for type 2 (non-insulin-dependent, maturity onset) diabetes mellitus (3), and that flush susceptible diabetics may be less prone to develop late vascular complications during the course of their disease (4-6).

Flush reactions

The facial cutaneous blood flow is greater than in other cutaneous regions, which may account for the limited region of flushing. Facial vasodilatation is a result of increased activity in sympathetic fibres, originating in the spinal cord, with a second system originating in the brain stem. In addition other central neurons could be important in provoking and modifying the flush reaction (7).

Flushing is a relatively common phenomenon. In Table I some of its causes and possible mediators are listed.

Alcohol metabolism

Acetaldehyde is the first-step degradation product of ethanol, and alcohol dehydrogenase is the enzyme responsible. Acetaldehyde is further metabolized to acetic acid by aldehyde dehydrogenase (AldDH). The enzymes of alcohol and aldehyde metabolism exhibit a genetically determined heterogeneity, including isoenzymes and enzyme polymorphisms. Thus, alcohol metabolism varies from indi-

Table I. Clinical conditions and compounds associated with flushing, and postulated mediators (7).

Rosacea (- early symptom)	-
Systemic mastocytosis	histamine
Rubeosis diabetica	-
Red nose of alcoholics	-
Alcohol	acetaldehyde
Disulfiram-alcohol	acetaldehyde
	dopamine β -hydroxylase
Disulfiram-alcohol-like reactions	acetaldehyde
Morphine	-
Carcinoid syndrome	bradykinin
	histamine
	serotonin
Neoplasia	histamine
	vasoactive intestinal peptide
	prostaglandins
	calcitonin
Fermented-alcohol	tyramine, histamine
Menopause	central catecholaminergic system
Glutamate	acetylcholin-like substance
Substance-P	-
Thermal-induced	heat

vidual to individual (8). The enzymes are NAD^+ dependent (9). Hepatic AldDH activity has been shown to be rate-limiting for the oxidation of acetaldehyde in the liver (10). The greater proportion of total hepatic AldDH activity is found in the mitochondrial fraction, while a smaller proportion is found in the cytosolic fraction (11). Acetaldehyde has several biochemical effects: It has been shown to diminish cell proliferation of rabbit aortic myocytes in culture (12); by competing with NAD^+ it inhibits AldDH, and the oxidation of ethanol reverses the NAD^+/NADH ratio, both of which reactions cause an alteration in the metabolism of serotonin (9) and possibly in the degradation of other aldehydes of biogenic amines (8).

Alcohol flush

It was reported by Wolff (13) that the greater alcohol sensitivity of Mongoloids as compared with that of Caucasoids was related to variations in autonomic reactivity, and not due to slower alcohol metabolism. In Japanese who experienced alcohol flushing, however,



Mizoi et al (14) showed an increase in blood acetaldehyde concentrations that was lacking in non-flushers. During the last decade various explanations for the increase in blood acetaldehyde concentrations in Mongoloids after alcohol drinking have been put forward. Harada et al (15) suggested delayed oxidation of acetaldehyde, rather than increased production, as causing the flush reaction in Japanese after alcohol intake. In 1981 Agarwal et al (16) showed that, due to lack of the low K_m isoenzyme of AldDH, Japanese tend to have difficulty in metabolizing acetaldehyde. In contrast, they found no direct relationship between atypical alcohol dehydrogenase and the flush phenomenon.

A variety of potentially vasoactive pharmacological agents are found in alcoholic beverages such as sherry and beer (7). For example, it may be the histamine present in the sherry ingested by subjects that was in fact responsible for flushing, which was blocked by histamine antagonists (17,18). Thus, fermented alcoholic beverages such as sherry are to be avoided in studies on alcohol flushing.

Disulfiram alcohol flush

While the alcohol-induced flush and the disulfiram-alcohol-induced flush shares several clinical characteristics, the disulfiram-ethanol reaction may be more complex, and several mechanisms have been proposed (7):

1. Acetaldehyde accumulation,
2. Production of a toxic quaternary ammonium compound from disulfiram and ethanol,
3. Synergistic toxic action of disulfiram and accumulated free alcohol,
4. Serotonin - liberation and decreased degradation,
5. Mast cell degranulation,
6. Decreased conjugation of toxic endogenous compounds by inhibition of the glucuronic-acid-conjugating system,
7. Inhibition of catecholamine synthesis at the dopamine β -hydroxylase step.

The intensity of the disulfiram-ethanol reaction is dependent on the blood acetaldehyde concentration and dopamine β -hydroxylase activity. With the disulfiram-induced inhibition of dopamine β -hy-

droxylase, and the decreased content of norepinephrine, acetaldehyde causes vasodilatation (19). Low dopamine β -hydroxylase concentrations also appear to predict susceptibility to adverse reactions to disulfiram (20).

Several agents have been reported to produce a reaction resembling the disulfiram alcohol flush: calcium carbamide, phentolamine, griseofulvin, metronidazole, sulfonyleurea, cephalosporins, various industrial agents and mushrooms (7).

Chlorpropamide alcohol flush

In the chlorpropamide alcohol flush (CPAF), there is a visible flush of the face and neck associated with a sensation of facial warmth. The flush is seldom distressing to the patient although it may be mildly embarrassing. More severe cases are accompanied by breathlessness, wheezing, tachycardia and injection of the conjunctive. In people on chlorpropamide (CP) therapy, the reaction normally begins within 15 min of their drinking alcohol, reaching a peak in about 20-30 min, after which it usually subsides, although it may last for as long as 1-2 hours. All brands of alcohol will provoke the reaction in susceptible individuals and even very small amounts of alcohol is often sufficient. This side effect of CP begins within 2-5 days of therapy being started, and persists until 4-7 days after medication is stopped (21).

Clinically it is rare to find alcohol flushing in patients taking other sulfonyleurea drugs, though it occasionally occurs in conjunction with tolbutamide (22,23).

CPAF was first reported by Bertram et al (21) in 1956, within a year of the introduction of the sulfonyleureas. During a symposium on chlorpropamide held at the New York Academy of Science in 1958 (24), several of the papers presented mentioned an alcohol flush reaction in patients on CP therapy: Signorelli reported an alcohol flush reaction in 34% of patients on CP, which promptly disappeared when daily wine drinking was stopped and reappeared in response to subsequent alcohol challenge (25); Scaledo and Borges reported an instance in one patient of a manifestation resembling mild lung oedema which disappeared when the alcohol intake was stopped (26); and Daeppen et al reported the occurrence of respiratory oppression in

patients whose alcohol flush reaction was more pronounced than others' (27).

A Frech group at first suggested CPAF to be a postprandial vasomotor phenomenon (28), but soon realized that the effect was not produced by food but by the alcohol taken with it (29). FitzGerald et al reported CPAF in 33% of patients on CP therapy, and concluded that it was a very common side effect but that patients probably preferred not to mention it (30).

Pharmacokinetics and biotransformation of chlorpropamide

P-chlorophenylsulfonylpropylurea (CP) is a sulfonylurea compound with hypoglycemic action. Orally administered, more than 95% of given dose of CP is absorbed (31). The time required to reach peak concentration varies between one to seven hours (mean absorption half-life of 0.5 hour), while the half-life of the drug in plasma is 33 hours (range 25-42) after one tablet of 250 mg CP (32). The drug distribution correlates to the extra-cellular space in man and about 85% of CP is albumin-bound in plasma (33). There is a correlation between the dosage given and plasma concentrations of the drug (34,35,36) with a wide variation between individuals (37). CP determination with high-pressure liquid chromatography measures the total unchanged CP concentration, but not that of its metabolites (38). CP is metabolized to CBSU (p-chlorobenzensulfonylurea), 2-hydroxy-CP and 3-hydroxy-CP. CBSU is further metabolized to CBSA (p-chlorobenzensulfonamide). Taylor (31) demonstrated higher concentrations of the metabolites in urine than in plasma. For example, 55% of recovered CP from the urine over a period of 24 hours and 1.5% from plasma after 2 hours is hydroxy CP. Of the CP administered, over 95% is excreted in the urine, of which about 20% is unchanged (32).

MATERIAL AND METHODS

Patients and normal subjects (papers I-VII)

Those studied were 189 type 2 diabetics, 75 females and 114 males, from the Diabetic Care Unit at the Department of Medicine in Malmö. The median age was 65 years (range 16-83), the mean duration of disease after the diagnosis of diabetes was 8 years (range 1-22), and the mean body weight was 117% (range 85-160) of the ideal. Thirtyone patients were on regular CP therapy (papers I,II,IV), 78 patients were on other sulfonylurea drugs (glibenclamide or glipizide), and 24 patients, after several years on peroral anti-diabetic drugs, were on insulin treatment. The remaining 56 patients were on diet only. Healthy subjects, 12 females and 54 males, mean age 54 years (range 46-60), were used as controls. Detailed data are given in papers I-VII.

Clinical examinations (papers II, VII)

Each proband was carefully questioned as to any occurrence of diabetes mellitus among parents, siblings, or children, and interviewed with regard to smoking and alcohol habits, history of ischemic heart disease (angina pectoris and/or myocardial infarction), intermittent claudication, cerebral infarction and the use of drugs. Patients in paper VII and control subjects answered a questionnaire including nine questions relating to alcohol consumption (Mm-MAST), excessive alcohol consumption being considered a possibility when two or more affirmative answers were given (39).

Systolic and diastolic blood pressures were taken by sphygmomanometer on the right arm, with the patient supine and after 5 min rest. The retina was examined by ophthalmoscopy in mydriasis. Foot pulses (dorsalis pedis and post-tibial pulses) were examined by palpation and by an ultrasonic surface blood flow detector (Vasculascope, Kamiya Tsusan Kaisha Ltd., Tokyo). Vibration perception thresholds were determined over the medial malleoli using a Bio-Thesimeter (Bio-Medical Instruments Co., Newbury, Ohio), the result being expressed in volts as the mean of the left and right ankle values. All clinical examinations were made by one and the same examiner.

To facilitate comparison of the extent of diabetic complications and other variables, systems of scoring were evolved to rank macro-angiopathy, micro-angiopathy and peripheral neuropathy. Having been designed to surpass the micro-angiopathy and peripheral neuropathy scores in importance, the macro-angiopathy score was predominant in the total complication score. Detailed data are given in paper VII.

CPAF challenge test (papers I-VII)

The chlorpropamide alcohol flush test was carried out essentially as described by Leslie and Pyke (3), except that, pure ethanol (8g) in fruit juice was used instead of sherry. Patients on regular CP therapy received their normal dose (125-375 mg) in the morning of the test day. The majority of subjects, not being on CP treatment, were given a single dose of 250-375 mg CP (Diabinese, Pfizer Corp.) in the evening 12 hours before the test. All other sulfonyl-urea medication was stopped for 24 hours, but probands had breakfast 2-3 hours before the test. The patients, 4-5 at the same time, were studied in a room with an ambient temperature of between 20 and 23°C, which was constant for the duration of the test procedure. Facial skin temperature was measured 2 cm below the lateral canthus of the left eye, readings being taken every 5 min, starting 20-30 min before alcohol intake (the interval usually required for adjustment), and continuing 30 min after ingestion. Blood samples for measurements of ethanol, acetaldehyde and chlorpropamide concentrations were obtained by venepuncture immediately before alcohol intake and 25 min afterwards - the point at which flushing reached a peak in most subjects (paper I, 40). The appearance or absence of facial flush was judged by one and the same examiner at all test occasions, without knowing the result of the skin temperature measurement.

Facial skin temperature measurements (papers I-VII)

The increase in facial skin temperature (ΔT) was the method first used to study the flush phenomenon objectively (41). A thermocouple skin applicator was connected to a galvanometer (Electrolab., Copenhagen), and adjusted before use on every occasion. In paper III the results of the skin temperature measurements were also converted into "per cent of maximum possible temperature rise" (%T) and "increase in malar thermal circulation index" (ΔMTCI):

$\Delta T = T_{30} - t_0$, where T_{30} is the skin temperature 30 min after alcohol ingestion and t_0 is the baseline temperature (6),

$\%T = (T_{30} - t_0)/(36.5 - t_0) \times 100$, where 36.5°C is the maximal possible normal facial skin temperature (42),

$\Delta\text{MTCI} = (T_{30} - 22) \times (37 - t_0)/(37 - T_{30}) \times (t_0 - 22)$, where 22°C is the mean ambient temperature and 37°C the core temperature (43).

The following definitions of a positive test (a flush) were used:

$\Delta T \geq 1.0^\circ\text{C}$ (44,45), $\%T \geq 24\%$ and $\Delta\text{MTCI} \geq 1.5$ (43).

Blood (plasma) acetaldehyde and ethanol (papers I-VII)

Acetaldehyde was derivated with semicarbazide using a modification of the method described by Stowell (46). Blood samples were collected in 10-ml vacuum tubes containing 3 ml buffered semicarbazide solution, freshly prepared for every trial to avoid artifactual acetaldehyde formation. Blood cells were separated in graded tubes, and 2 ml of the diluted plasma samples thus obtained was transferred to precooled 25-ml (papers I-VI) or 4-ml (paper VII) glass vials. Seventy per cent perchloric acid (0.1 ml) was added to hydrolyze the acetaldehyde semicarbazone. Tert-Butanol was used as internal standard. The glass vials were sealed with rubber membranes and incubated at a temperature of 65°C for 20 min (papers I-VI) or 60°C for 30 min (paper VII). Five ml (papers I-VI) or two ml (paper VII) of the head-space was injected in a Varian 2400 gas chromatograph equipped with flame ionization detector. A Porapak Q column, kept at 110°C (papers I-VI) or 115°C (paper VII), was used with helium as the carrier gas. The acetaldehyde and ethanol concentrations were thus determined in plasma (papers I-V) or in blood (papers VI-VII). In paper VII the samples had been frozen for a month before acetaldehyde concentrations were measured.

CPAF Criteria (paper II) and CPAF Index (paper V)

In order to assess more objective evidence of CPAF than the mere occurrence of a visible facial flush, three CPAF criteria and a CPAF Index were evolved. The basic or primary CPAF criterion, a visible facial flush, and two additional CPAF criteria were chosen. In previous reports on CPAF testing, either a ΔT value $\geq 1.0^\circ\text{C}$ (but usually under 5°C), or a ΔP -acetaldehyde value $\geq 4 \mu\text{mol/l}$ (but usually less than $20 \mu\text{mol/l}$), was taken to indicate the presence of a flush reaction (44,45, paper I), thus defining the second and

third CPAF criteria, respectively (paper II). The CPAF Index was independent of the visibility of the flush and, being calculated as the sum of the ΔT value and a quarter of the ΔP -acetaldehyde value, thus rendered the two values equal in importance (paper V).

Alcohol challenge test (papers I, II, IV, VII)

In subjects who were positive on the single-dose CPAF test, a similar test (but without CP premedication) was performed more than 10 days after the first test.

Plasma chlorpropamide (papers IV, V, VII)

A high-pressure liquid chromatographic (HPLC) technique was used for measuring plasma CP concentrations, tolbutamide serving as the internal standard (38).

Erythrocyte aldehyde dehydrogenase activity (papers VI-VII)

The activity of aldehyde dehydrogenase in erythrocytes was assessed by the method of Inoue et al (47), modified as follows. The blood samples were collected in heparinized vacuum tubes, kept at 4°C, and centrifuged at 1000 x g for 10 min at 4°C within an hour of collection. A mixture in fixed proportions of plasma and erythrocytes (5:1) was homogenized by freezing (-70°C) and thawing. Three ml of the homogenate was incubated (37°C) with 100 μ mol acetaldehyde/l and one mmol nicotinamide-adenine dinucleotide (NAD^+)/l in 4-ml airtight glass vials. Samples were taken at fixed intervals and the reaction stopped by cooling the vials in iced water for 10 min. Deproteinization with 0.5 ml 35% perchloric acid followed. After centrifugation (at 4°C), the clear supernatant was assayed for acetaldehyde. The acetaldehyde was measured by head-space gas chromatography as described above, except that diethylether served as internal standard in paper VII.

Other chemical analyses (paper VII)

S-Creatinine, fasting S-Cholesterol, fasting S-Triglycerides, random B-Glucose, and Erythrocyte HBA_{1c} were analyzed by routine hospital methods. Urine albumine excretion was determined by Albstix.

Statistical evaluation (papers I-VII)

Where their distribution was skewed, raw data were subjected to logarithmic transformation prior to statistical analysis. For correlations, the method of least squares and Spearman rank correlation coefficient were used. The Wilcoxon signed rank test (paper VI) and the Mann-Whitney U-test (paper VII) were used to assess AldDH activity. Otherwise unpaired t-test and chi-square analysis were used to assess the statistical significance of differences. $P < 0.05$ was considered significant. Results are given as mean $\pm$ SEM.

RESULTS AND DISCUSSION

METHODOLOGICAL STUDIES (PAPERS I-III)Objective methods of studying CPAF*Facial skin temperature*

The mean basal facial skin temperature (t_0) was the same in subjects with a visible flush (flushers) and those without (non-flushers), though females (both CPAF positive and negative) had a lower t_0 than males ($p < 0.001$) (Table I in paper II). The highest facial skin temperature measured after alcohol ingestion was 36.2°C . After calculations of respective facial skin temperature measurement the following results were obtained for 57 flushers and 76 non-flushers, respectively: ΔT , $1.7 \pm 0.1^\circ\text{C}$ and $0.6 \pm 0^\circ\text{C}$; %T, $40.0 \pm 2.2\%$ and $14.9 \pm 1.0\%$; and ΔMTCI 2.1 ± 0.2 and 1.2 ± 0 ($p < 0.001$, Fig 1 in paper III). Using the three definitions described in methods, the specificity and sensitivity of a positive test with respective temperature method was 88, 86 and 96%, and 90, 86 and 86%, respectively (paper III). The reproducibility of the skin temperature measurements was acceptable (Table II).

Table II: Skin temperature measurements expressed as ΔT (temperature increase) during repeated CPAF tests in 9 diabetic patients.

<u>Female flushers</u>		<u>Male flusher</u>	<u>Male non-flushers</u>
1) 4.5	4) 1.4	7) 0.9	8) 0.1
4.8	1.0	1.1	0.1
5.2	1.1	1.0	-0.1
5.1		1.2	0.5
5.3	5) 1.7		
5.0	1.4		9) 0.6
	1.5		0.5
2) 1.3	6) 1.8		0.6
1.3	2.8		0.7
1.7	1.6		
1.3			
1.8			
3) 1.4			
2.1			
1.8			
1.4			

Plasma acetaldehyde

Baseline plasma acetaldehyde concentrations were below or only just at a detectable level. The mean rise in plasma acetaldehyde concentration 25 min after alcohol ingestion (ΔP -acetaldehyde) was significantly higher ($p < 0.001$) in 51 subjects with a visible flush than in 27 non-flushers: $9.7 \pm 1.4 \mu\text{mol/l}$ and $2.6 \pm 0.2 \mu\text{mol/l}$, respectively (Fig 2 in paper II). Furthermore, ΔP -acetaldehyde correlated significantly ($p < 0.001$) to the intensity of the flush (ΔT) (Fig 1, paper IV). Using the definition of a positive test described in methods (ΔP -acetaldehyde $\geq 4 \mu\text{mol/l}$), plasma acetaldehyde determination had a specificity of 86% and a sensitivity of 85%.

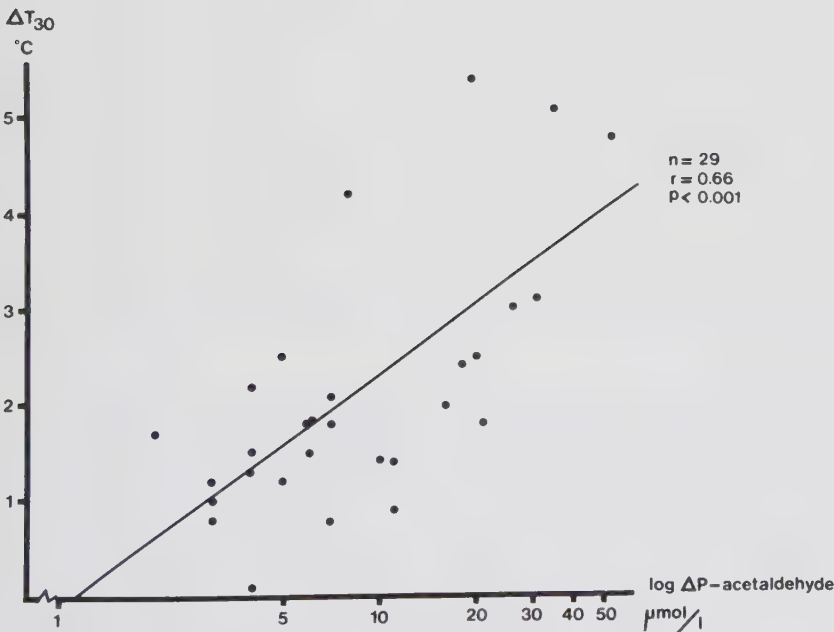


Fig 1. Skin temperature increase at 30 min (ΔT) versus log of difference in plasma acetaldehyde in patients on regular chlorpropamide therapy.

CPAF Criteria and CPAF Index

By definition, the basic CPAF criterion was fulfilled by all 73 flushers and by none of the 64 non-flushers (paper II). The second CPAF criterion ($\Delta T \geq 1.0^{\circ}\text{C}$) was satisfied by 90% of the flushers and by 14% of the non-flushers, while the third criterion ($\Delta \text{P-acetaldehyde} \geq 4 \mu\text{mol/l}$) was satisfied by 86% of the flushers and by 15% of the non-flushers; of the flushers, 78% fulfilled all three criteria and 22% fulfilled two criteria, while 74% of the non-flushers failed to satisfy any criteria and the remaining 26% fulfilled only one. Thus, there was no overlap between flushers and non-flushers.

With the exception of one proband with no visible facial flush, the CPAF Index unequivocally distinguished flushers from non-flushers (Fig 2 in paper V).

Alcohol challenge test

Alcohol challenges, without CP premedication, were performed in 41 of the 45 subjects with a visible flush on the single-dose CPAF test (paper II). Values of ΔT were lower during alcohol challenge tests ($0.8 \pm 0.1^{\circ}\text{C}$) than during CPAF tests ($1.9 \pm 0.2^{\circ}\text{C}$) ($p < 0.001$), as were those of $\Delta \text{P-acetaldehyde}$, 1.9 ± 0.3 and $7.4 \pm 1.3 \mu\text{mol/l}$, respectively ($p < 0.001$). In 14 subjects (34%) there was alcohol flushing and $\Delta T \geq 1.0^{\circ}\text{C}$. Plasma acetaldehyde concentrations did not exceed $4 \mu\text{mol/l}$ during any alcohol test. At the foregoing CPAF tests, $\Delta \text{P-acetaldehyde}$ values had been below $4 \mu\text{mol/l}$ in four of the 14 alcohol flushers, and had been $4 \mu\text{mol/l}$ or more in the remaining 10 subjects.

Prevalence of CPAF

Visible facial flushing during CPAF challenge tests was observed in 85 of 164 (52%) type 2 diabetics (papers II, III, IV). CPAF correlated neither to age nor to duration of disease after diagnosis. Of the 31 on regular CP therapy, 28 (90%) flushed, while 57 of 133 (43%) single-dose CPAF tested patients flushed ($p < 0.001$). Results of single-dose CP challenge tests did not vary whether the dose was 250 mg, when 42% of the probands flushed, or 375 mg, when 43% of them flushed. Of 35 control subjects, 5 (14%) flushed in response to single-dose CPAF tests (paper II). The interpretation of the combined results of CPAF and alcohol challenge tests is discussed below.

Discussion

The first studies on CPAF were based on the subjective assessment of flushing (3). A 91% agreement was found between subject and observer assessments as to the presence or absence of a flush (48). Thermography has been shown to be useful in distinguishing between flushers and non-flushers (49), but the method most commonly used to assess CPAF has been skin temperature measurement.

Facial skin temperature

Both basal skin temperature (t_0), and increments in temperature (ΔT), may be affected by ambient temperature, emotional factors, smoking before the test, or other unknown variables. The reproducibility of skin temperatures was found to be limited in a study by Köbberling et al (50). De Silva et al reported a significantly negative correlation between t_0 and peak increment in skin temperature after 40 ml sherry and pretreatment with CP (40), which has since been confirmed in several other studies (51,52,53,54). Wilkin (43) reported the baseline facial temperature to be lower in type 2 diabetics (especially those who were CPAF positive) than in non-diabetic subjects, and speculated that this may be due to diabetic autonomic neuropathy. Loss of innervation of the vasodilator fibers of the cutaneous vasculature would lead to relative vasoconstriction and a lower t_0 . However, this explanation was rejected since CPAF was seen in non-diabetics and was more common in mild diabetes unaccompanied by subjective symptoms or clinical signs of autonomic neuropathy (55).

Pyke's group (49) reported that with similar t_0 flushers showed an increase in skin temperature after CP and alcohol three times greater than that of non-flushers. After a single dose of CP, the mean increase in facial temperature (0-40 min after ethanol) was significantly higher in CPAF positive than CPAF negative subjects, though there was some overlap between the two groups. After two weeks on CP medication, during alcohol challenge the mean increase in temperature rose in the CPAF positive subjects but remained unchanged in the CPAF negative subjects, and there was no overlap between them (56). Pontiroli et al (57) found no influence of the basal skin temperature on the alcohol-induced temperature rise. An increase in skin temperature of 1°C discriminated between patients with and

without CPAF. Groop (58) defined CPAF positivity as an increase in facial temperature $\geq 1.5^{\circ}\text{C}$ 30 min after alcohol intake, or a peak $\geq 2.0^{\circ}\text{C}$. He found the variation coefficient to be 18%, t_0 to be significantly lower, and rise in temperature to be significantly higher in the flushers than in the non-flushers ($p < 0.001$).

Radder et al reported a considerable overlap in peak increase in skin temperature between flushers and non-flushers (59). Toeller and Gries (60) reported poor correlation between facial temperature increase and visual flushing. The flush and subjective symptoms were provoked irregularly, whereas the temperature measurements (0-75 min after 40 ml sherry) proved objective and the results reproducible.

In the present study (paper II), while no difference in mean t_0 was found, ΔT significantly differed between flushers and non-flushers, 32.1 ± 0.1 versus $32.5 \pm 0.2^{\circ}\text{C}$, 1.9 ± 0.1 versus $0.6 \pm 0^{\circ}\text{C}$, respectively. It is clear that subjects whose basal temperature is already high ($>34.5^{\circ}\text{C}$) may flush without a noticeable increase in skin temperature. Conversely, an increase in temperature may occur without a visible flush (61). Nevertheless, the ΔT method had a specificity of 88% and a sensitivity of 90%, (paper III). The finding by Pyke et al (55) and De Silva et al (40) that the temperature increase during the CPAF reaction occurs sooner (20-30 min) than during the simple alcohol flush (50 min) is perhaps the strongest reason for only measuring skin temperature increases between 0 and 30 min. Wilkin (43) introduced the method of malar thermal circulation index (ΔMTCI), derived from the relationship between skin temperature and cutaneous blood flow. In spite of being more physiological, neither ΔMTCI , nor the %T method introduced by Wiles and Pyke (42), proved to be more specific or sensitive than the ΔT method. Wiles and Pyke have since stated that flushers have a %T $\geq 35\%$ (48); applying this definition of a flush to our material, the specificity would be nearly 100% but the sensitivity would decrease markedly (paper III).

Plasma acetaldehyde

The plasma acetaldehyde concentration proved to be an objective reflection of the CPAF; indeed, increase in plasma acetaldehyde even correlated to intensity of the flush reaction (paper IV). Although the ethanol ingestion was 4-6 times greater and tolbutamide was

given in a very large dose in Büttner's study from 1961 (62), our results matched his. He determined plasma acetaldehyde concentrations with a spectrophotometer after an enzymatic analysis. In 1962, FitzGerald et al (30) were unable to find differences in blood acetaldehyde concentrations following ethanol ingestion, whether or not CP treatment was given. Acetaldehyde was measured in whole blood samples by using a colorimetric method after distillation into bisulfite. Stowell's method (46) proved capable of determining even low plasma acetaldehyde concentrations, and the interference from ethanol (a major problem encountered with the earlier methods) was negligible. The difference in acetaldehyde concentrations between CPAF positive and CPAF negative type 2 diabetics, first reported by us (63), was confirmed by Pyke's group (56) and by Groop et al (64); to determine the blood acetaldehyde concentration, Pyke's group used the method of von Wartburg and Ris (65) and Groop et al used the method of Eriksson et al (66).

CPAF Criteria and CPAF Index

The CPAF Criteria and CPAF Index were designed to enhance the sensitivity and specificity of the methods. With either combination of methods it was possible to evaluate the CPAF test objectively in all but one proband. In accordance with these results, Karamanos et al (44) compared the subjective feeling of flush (subjective criterion of a positive CPAF test) with an increase in skin temperature $\geq 1.0^{\circ}\text{C}$ (objective criterion) in 48 diabetics on CP treatment, and reported matching results in 79% of the patients.

Alcohol challenge test

Although Köbberling et al (50) introduced the placebo-alcohol test in 1980 he did not perform enough placebo tests to obtain prevalence figures for alcohol flushing. De Silva et al (40) reported placebo-alcohol flushing in 9 of 11 CPAF positive diabetics on diet only. Micossi et al (67) reported 32% of 108 type 2 diabetic patients to flush on CPAF tests. A positive placebo-alcohol test result was found in 15% of the 108 patients. In 239 type 2 diabetic patients, Ng Tang Fui et al (68) reported the prevalence of a flush response during alcohol challenge to be 10% after CP but no placebo, 17% after CP and placebo, and 5% after placebo but no CP. All these

three research teams suggested true CPAF to exist only if placebo-alcohol flushing was absent.

Jefferys et al (69) reported a clearly alcohol dose dependent rise in facial skin temperature in alcohol flushers, while the CPAF positive diabetics had a full hyperemic response already on a small (8g) standard alcohol dose. Groop et al (64) found a significantly higher flush score (=intensity), increase in facial skin temperature, heart rate and blood acetaldehyde concentrations after CP than after placebo during alcohol challenge tests. In contrast to the suggestion by Jefferys (69), Groop reported these differences to be accentuated after higher alcohol doses. According to Pyke's group (55), patients distinguish clearly between CPAF and the common alcohol flush. CPAF comes on sooner and is more intense than the alcohol flush.

Disregarding difference in intensity, in our experience the facial flush on alcohol challenge, either with or without CP premedication, seems almost identical to the observer, as well as to the proband. Indeed, the two reactions could partly be mediated by the same mechanisms (see biochemical studies below). In the present study, 34% of CPAF positive diabetic patients also experienced alcohol flushing (paper II). Increased acetaldehyde concentrations, characteristic of the CPAF reaction, were absent during the simple alcohol flush reaction - a finding confirmed both by Barnett et al (56) and Groop et al (64). In most subjects a rise in acetaldehyde concentration (an effect of CP-induced inhibition of AldDH) is necessary to induce the flush reaction. In some subjects (the alcohol flushers), the mechanisms leading to facial flushing can be triggered by alcohol alone without detectable increases in the acetaldehyde concentration. Their acetaldehyde concentration having increased during the CPAF test, 10 of the 14 alcohol flushers in paper II were consequently both CPAF positive and alcohol positive, the remaining 4 alcohol flushers being false CPAF positive. Thus, 5% of patients were not CPAF positive according to the CPAF test, but CPAF negative diabetics with the ability of alcohol flushing. Consequently, the reported prevalence of CPAF should be reduced by about 5 per cent.

Prevalence of CPAF

Considerable variation exists in the reported prevalence of CPAF in type 2 diabetic patients: mean 33%, and range 17-52% (Table III). Ethnic idiosyncrasy might be one possible explanation to these discrepancies, and the methods used to assess the flush might be another. As discussed below, one reason for the high prevalence of CPAF, both in this and in other studies, could be the number of flush positive diabetics on maintenance CP therapy. The prevalence of CPAF was reduced from 52% to 43% using single-dose tests only, though even the latter figure is still high. By correcting the figures with regard to the prevalence of alcohol flushing, the prevalence of CPAF could probably be reduced by 5% as described above. In type 1 diabetics and in control subjects, the prevalence of CPAF has been less controversial: mean 20% (range 0-30%) and mean 13% (range 7-17%), respectively.

Table III. Prevalence of CPAF in type 2 and type 1 diabetics, and in control subjects. The CPAF tests were made with a variation in CP premedication dose from 250 mg 12 hours before the test to 500 mg of CP as daily anti-diabetic therapy. The results were not influenced by placebo-alcohol tests.

Reference	Type 2 diabetic patients		Type 1 diabetic patients		Control subjects	
	Prevalence (%)	(N)	Prevalence (%)	(N)	Prevalence (%)	(N)
FitzGerald et al (30)	22	100	-	-	-	-
Leslie & Pyke (3)	51	234	10	60	10	60
Köbberling et al (50)	17	145	23	437	17	154
Radder et al (59)	38	57	-	-	-	-
De Silva et al (40)	24	50	-	-	-	-
Panzram & Adolph (70)	20	40	-	-	-	-
Artensio et al (71)	25	195	0	30	-	-
Squadrito et al (45)	24	114	-	-	-	-
Micossi et al (67)	32	108	-	-	-	-
Lubetzki et al (52)	34	56	10	30	7	27
Zahr et al (72)	47	74	-	-	-	-
Groop (58)	38	160	-	-	-	-
Pontiroli et al (57)	21	97	30	33	-	-
Ng Tang Fui et al (68)	27	239	17	145	12	273
Present study	52	164	-	-	14	35
TOTAL	33	1833	20	735	13	539

PHARMACOLOGICAL STUDIES (PAPERS IV-V)

Body weight

The body weight of CPAF positive diabetics was lower than that of CPAF negative diabetics, 71.5 ± 2.5 kg and 77.9 ± 2.5 kg (NS), respectively, and that of females significantly than that of males (Table I in paper V).

Plasma chlorpropamide

Patients on regular CP therapy had significantly ($p < 0.001$) higher CP concentrations in plasma than those not previously exposed to it (Table IV, paper IV). Among subjects exposed to the single-dose CPAF test, females had higher CP concentrations than males ($p < 0.001$). Indeed, CPAF positive females and males, had significantly ($p < 0.05$) higher CP concentrations than CPAF negative females and males, respectively (Table II in paper V).

At single-dose CPAF tests, flushers had higher plasma CP concentrations than non-flushers: 34.6 ± 1.7 mg/l and 27.7 ± 1.3 mg/l, respectively ($p < 0.005$). However, all CPAF positive subjects on single-dose tests had lower CP concentrations than the three CPAF negative patients on regular CP therapy (Table IV, paper IV).

A significant negative correlation ($p < 0.001$) existed between body weight and plasma CP concentrations (Fig 2, paper V).

Significant positive correlations ($p < 0.05$ - 0.001) were found between plasma CP concentrations and both ΔT , ΔP -acetaldehyde, and CPAF Index values (Fig 3, Fig 3-6 in paper IV, Fig 2 in paper V). Alterations in CPAF reaction pattern followed changes in CP premedication doses: CPAF negative patients became CPAF positive, and vice versa (detailed data are given in paper IV).

Table IV. Plasma chlorpropamide concentrations (mean $\pm$ SEM) during CPAF tests.

	Number of patients tested	Plasma chlorpropamide [μ g/ml]				<i>p</i>
		CPAF-positive	<i>n</i>	CPAF-negative	<i>n</i>	
Single dose	74	34.6 ± 1.7	31	27.7 ± 1.3	43	< 0.005
Chronic therapy	31	92.2 ± 5.4	28	80.0 ± 17.1	3	NS

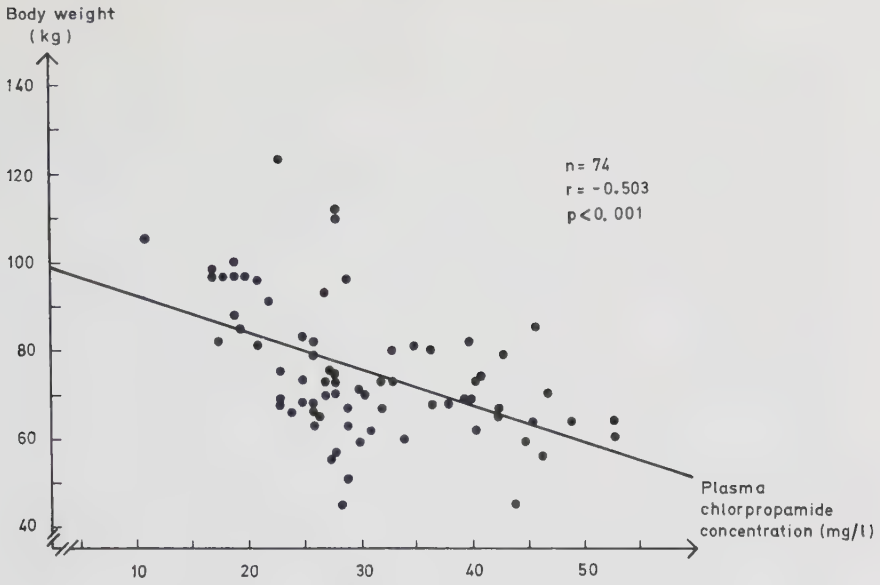


Fig.2 Correlation between body weight (kg) and plasma levels of chlorpropamide (mg/l) in 74 type 2 diabetics.

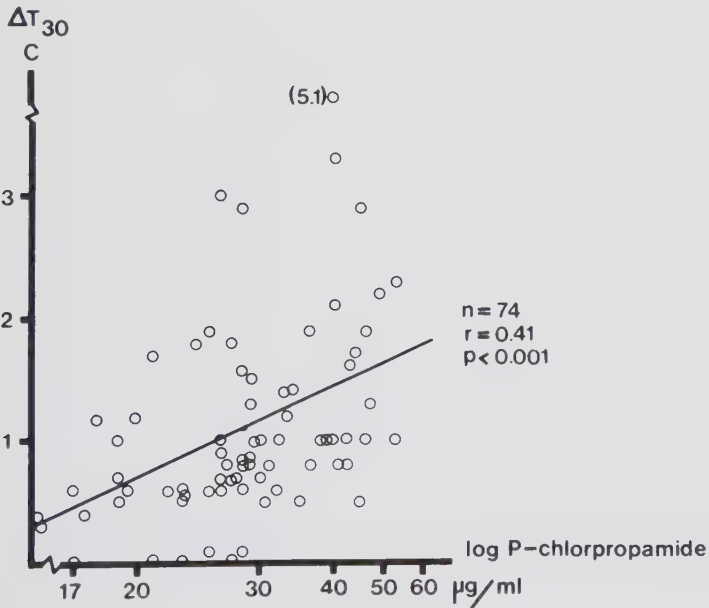


Fig.3 Difference in skin temperature at 30 min (ΔT) versus log serum concentration of chlorpropamide in patients given a single dose of chlorpropamide.

Sex differences

There was no difference in the prevalence of first-degree family history between CPAF positive and CPAF negative patients: 31/73 (42%) and 32/64 (50%), respectively (paper II). CPAF was more common in females than in males, but the difference was not statistically significant: 21/41 (51%) and 10/33 (30%), respectively (papers IV, V). Occurrence of alcohol flushing did not differ between the sexes (paper II).

Discussion

Genetic aspects on CPAF

Leslie and Pyke (3) suggested CPAF to be a dominant autosomal trait. Their reasons were several: one parent and half of the offspring of flushing type 2 diabetics were CPAF positive; identical twins showed the same reaction to the CPAF test, and direct transmission from parent to child through three generations was found in two families. Of the type 2 diabetics with a first-degree family history 81% were flushers, while of those without a family history only 31% had a positive CPAF test. Pyke and Leslie (73) also studied the offspring of diabetics in whom the disease had been diagnosed early in their lives, before the age of 30. Since this type of diabetes, maturity onset diabetes of the young (MODY), is dominantly inherited (74), presumably half of these offspring were genetically diabetic. Half of the offspring and all of the diabetics had positive CPAF test results. Pyke and Leslie therefore concluded that CPAF seems to precede the appearance of diabetes in these families. Similar results were obtained by Toeller and Gries, who reported six of eight MODY patients and nine of their 18 relatives to be flushers (60), although several other investigators have been unable to show that MODY was associated with a higher prevalence of CPAF (50,70, 75,76).

Fox et al (77) reported 32% positive CPAF tests in non-diabetics with a history of diabetes in first and second-degree relatives, compared with only 14% positive tests in controls without family histories of diabetes. Groop (58) found more first-degree family history of diabetes among flushers than in non-flushers. He also reported CPAF positivity to be characterized by increased frequency of HLA-A2 and decreased frequency of HLA-B7. But, in the present

study and in several other studies (50,52,59,67,68,71,78,79), CPAF positive patients did not have diabetes in the family more often than CPAF negative diabetics.

In an early study (6) we reported a significantly higher prevalence of CPAF in female than in male type 2 diabetic patients. This was soon confirmed by Micossi (80) and Radder et al (81), and later by other investigators (57,58,79,82). In the present study too, CPAF was more common in female than in male diabetics. However, in an epidemiological study of 239 type 2 diabetics, Ng Tang Fui and colleagues (68) did not find that women predominated among flushers. The finding of Micossi et al (67) that alcohol flushing without CP premedication was also more common in females than in males, is at variance with our own results (paper II). Recently, Pontiroli et al (83) reported CPAF to be more frequent in fast than in slow acetylators.

Body weight

Groop (58) reported that CPAF positive patients had a lower relative body weight than non-flushers: $119 \pm 3\%$ and $126 \pm 2\%$, respectively ($p < 0.05$). Furthermore, he showed that the uneven sex distribution between flushers and non-flushers disappeared when the alcohol dose was body-weight-matched (64). Neither Ng Tang Fui et al (68) nor Hillson et al (53) found any difference in body mass index between the two groups of CPAF reaction patterns. However, females had significantly lower body weight than males, and flushers had slightly lower body weight than CPAF negative patients (paper V, 84).

Plasma chlorpropamide

We reported higher chlorpropamide (CP) concentrations in CPAF positive than in CPAF negative diabetics (85). In addition, we found flushing to be more common among patients on regular CP therapy than among those previously unexposed to the drug and hence having lower concentrations at the time of the test (86). Simply by changing premedication dosages of CP, CPAF negative subjects were converted into CPAF positive, and vice versa (87). At first, Pyke's group disagreed that the concentration of CP in blood is critical for the outcome of the CPAF test, and claimed that the CPAF reaction could be satisfactorily elicited by a single chlorpropamide tablet (56).

However, they now believe that the size of the premedication CP dose affects the frequency of a positive response to alcohol challenge (49). There has been no evidence that differences in CP handling explain CPAF. Pontiroli et al (57) were able to determine serum concentrations both of CP and of one of its metabolites, p-chloro-benzenesulfonylurea (CBSU), each of which was similar in patients with and without CPAF. In the same study the percentage of flushing was 3% after 1 tablet and 34% after 4 tablets (2 tablets for 2 days) of 250 mg CP in type 2 diabetic patients. Other investigators have confirmed that, compared with single-dose CP, long term CP therapy increases the prevalence of CPAF (54,68,72).

The results of the present study confirmed our earlier findings. CPAF positivity was more common in patients on regular CP therapy than after single-dose CP premedication, plasma concentrations of CP were higher in flushers than in non-flushers (albeit with a considerable overlap between the two groups), and changes in CP dosage converted CPAF negative patients into CPAF positives and vice versa. Furthermore, the plasma CP concentration correlated directly to flush intensity, but inversely to body weight; indeed, the finding of higher CP concentrations in females than in males suggests that their lower body weight may account for the preponderance of females among flushers (84). Hillson et al (53) confirmed the existence of a positive correlation between increase in CP concentrations and rise of malar temperature. Therefore it was suggested that both CP and alcohol should perhaps be administered in dosages related to body weight to promote standardization of the CPAF test (88).

BIOCHEMICAL STUDIES (PAPERS I, IV, VI-VII)

Plasma ethanol

Only minimal concentrations of ethanol were found in plasma during CPAF tests, the highest being 8 mmol/l (= 0.4 per mille). The mean plasma ethanol concentration was significantly higher ($p < 0.005$) in CPAF positive than CPAF negative patients: 3.7 ± 0.3 mmol/l and 2.5 ± 0.3 mmol/l, respectively (Table II in paper IV).

Correlations between plasma concentration of acetaldehyde and chlor-propamide

At CPAF tests, there were significant correlations between P-acetaldehyde and plasma CP concentrations in subjects given a single-dose of CP ($p < 0.001$, Fig 4) and in patients on regular CP therapy ($p < 0.05$, paper IV).

Elimination of acetaldehyde in erythrocyte homogenates

The decline of the acetaldehyde concentration in erythrocyte homogenates was exponential with two phases (Fig 5, paper VI). The first phase was identical in CPAF positive and CPAF negative diabetics, whereas during the second phase (20-70 min) flushers had a significantly slower ($p < 0.005$) metabolism of acetaldehyde than non-flushers (Fig 3 in paper VI).

In one healthy male subject, repeated elimination curves were determined over a period of three weeks. The half-life of acetaldehyde (initial concentration 100 $\mu\text{mol/l}$) with between 20-60 min incubation was 85, 72, 51 and 73 min in the morning, 64 and 100 in the afternoon, and after 3 days with the subject on 5 mg of glibenclamide daily, the half-life was 100 min in the morning and 86 min in the afternoon. With an initial concentration of only 30 $\mu\text{mol/l}$, the half-life of acetaldehyde was significantly shorter ($p < 0.05$) than in the first experiments: 52, 50 and 58 min in the morning, 50 and 71 min in the afternoon, and after the glibenclamide treatment, 50 min in the morning and 75 min in the afternoon. The subject was asked to drink wine before one blood sampling and to take aspirin before another, but this failed to affect the results. The elimination curves showed more fluctuations between samples at 20, 30, 40, 50, and 60 min with initial acetaldehyde concentration of 30 $\mu\text{mol/l}$ than with one of 100 $\mu\text{mol/l}$. At 30 min, but not at 20 min, it was always clear that the second phase had been reached. In paper VII and in the healthy controls therefore, the initial concentration of acetaldehyde used was 100 $\mu\text{mol/l}$ and the elimination was determined with between 30 and 60 min of incubation (see clinical studies).

Aldehyde dehydrogenase activity in relation to blood acetaldehyde increase during CPAF

Blood acetaldehyde concentrations during CPAF tests were significantly higher ($p < 0.05$) in the lower AldDH activity quartile than in the higher AldDH activity quartile: $1.4 \pm 0.3 \mu\text{mol/l}$ and 0.6 ± 0.1

$\mu\text{mol/l}$, respectively, subjects with plasma concentrations of CP in excess of 1 SD from the mean having been excluded (paper VII).

Alteration in aldehyde dehydrogenase activity after chlorpropamide exposure

In 20 individuals (of whom 11 had vascular disease, 7 were females, 6 were CPAF positive and none was suspected of alcohol overconsumption, mean basal T1/2 of acetaldehyde of 136 ± 18 min), the AldDH activity was determined 12 hours after oral exposure to 375 mg of CP. There was a significant ($p < 0.05$, paired t-test) decrease in enzyme activity: mean T1/2 of acetaldehyde after CP was 222 ± 40 min. However, at CPAF testing no correlations were found between plasma concentration of CP (range 39-53 mg/l) and reduction of AldDH activity, or between the reduction of AldDH activity and blood acetaldehyde increase (unpublished data).

Discussion

Plasma ethanol

Pyke's group stated that the size of the alcohol dose is immaterial in CPAF (89). Jefferys et al (69) showed that a small dose sufficed to elicit CPAF, and that, in sharp contrast to simple alcohol flushing, CPAF was not intensified by larger alcohol doses. Significantly higher plasma ethanol concentrations were reported in flushers than in non-flushers (paper IV). Theoretically, this difference could be due either to the direct inhibition of alcohol dehydrogenase by CP, or to acetaldehyde accumulation. However, Groop et al (64) demonstrated that type 2 diabetic patients, having been differentiated as CPAF positive or negative using a single-dose CPAF test, changed in CPAF reaction pattern after adjustment of the alcohol dose in relation to body weight; they concluded that body-weight-matching of the alcohol dosage is essential in CPAF testing.

Chlorpropamide inhibits aldehyde dehydrogenase activity

In 1962 Larsen and Madsen (90) reported on the inhibition of ethanol metabolism in cats by tolbutamide. Since only the disappearance rate of ethanol was reported, it is unclear whether only the alcohol dehydrogenase, or the aldehyde dehydrogenase (AldDH) was inhibited, or both. Czyzyk and Mohnike (91) were unable to find alterations in alcohol disappearance rate after tolbutamide, but a year later Deitrich and Hellerman (92) showed that CP, but not CBSU

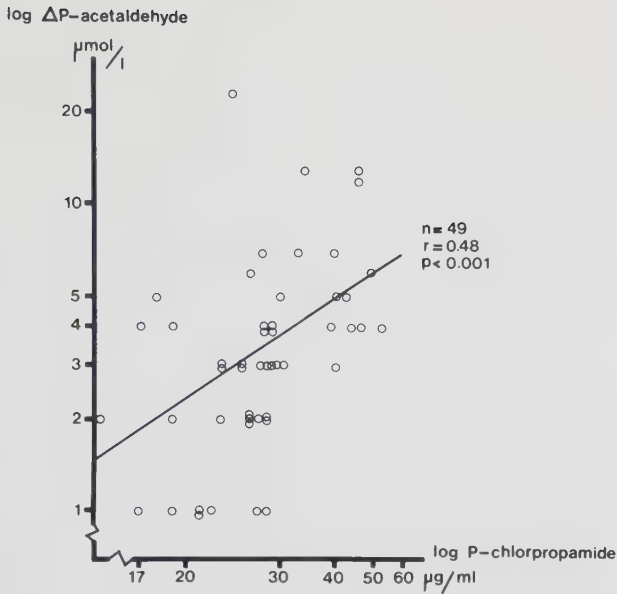


Fig.4. Log of difference in plasma acetaldehyde versus log plasma concentration of chlorpropamide in subjects exposed to a single dose of chlorpropamide.

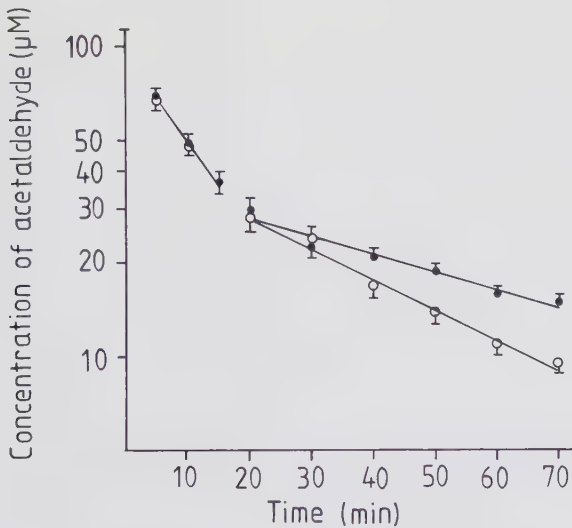


Fig.5. Elimination of acetaldehyde (initial) concentration $100 \mu\text{mol/l}$ in an erythrocyte homogenate with $1 \text{ mmol NAD}^+/\text{l}$. Two regression lines were calculated representing the two phases of acetaldehyde elimination, from 5-15 min and from 20-70 min, respectively (mean $\pm$ SEM). Flushers = $\bullet$, non-flushers = $\circ$.

and CBSA, inhibits AldDH activity; inhibition of AldDH by CP, competing with NAD^+ , was 25% as compared with 58% inhibition by disulfiram (Antabuse). Deitrich and Hellerman suggested that the "disulfiram effect" may not be solely attributable to the accumulation of acetaldehyde. Accumulation of the other aldehydes is also possible, since liver aldehyde dehydrogenase is a rather non-specific enzyme, oxidizing many aromatic and aliphatic aldehydes, and disulfiram is a non-specific inhibitor of several enzymes.

Our present results clearly indicate that the accumulation of acetaldehyde in blood during CPAF is correlated to the concentration of CP, both in regular therapy and after single-dose exposure (paper IV). These results have since been confirmed by Groop et al (64).

Aldehyde dehydrogenase activity

Acetaldehyde binds to plasma proteins (65), erythrocytes (93), and to phospholipids (94). The acetaldehyde-protein binding can be broken by perchloric acid (65,93). No difference in protein binding was found in 7 flushers and in 5 non-flushers (unpublished work). Elimination of NAD^+ by dialysis of erythrocyte homogenates completely inhibited the elimination of acetaldehyde. After NAD^+ was added the AldDH activity was restored. The adding of 5 μM disulfiram induced a 91% inhibition of the enzyme (47). We have confirmed these results (unpublished work), but we have not studied whether or not individual differences in non-enzymatic breakdown of acetaldehyde were present. In blood homogenates with no added NAD^+ , acetaldehyde was promptly eliminated during the first minute, probably as a result of protein binding. After the first minute no elimination of acetaldehyde could be measured (95).

Harada et al (96) studied hair root lysates for low K_m AldDH isoenzyme. This isoenzyme of liver AldDH was absent in about 50% of Japanese, and only these subjects showed facial flushing after drinking alcohol. Harada et al found a correlation between flushing and raised blood acetaldehyde concentrations in connection with AldDH deficiency, which agrees with our own finding that blood acetaldehyde concentrations during CPAF tests were higher in subjects with low AldDH activity than in those in whom it was high (paper VII). Towell et al (97) demonstrated that erythrocyte AldDH

activity was almost completely extinguished after the initiation of disulfiram (Antabuse) treatment. Recovery of AldDH activity after the discontinuation of disulfiram treatment took 3-4 months indicating de novo protein synthesis of erythrocytes.

Our finding of a biphasic acetaldehyde metabolism in erythrocyte homogenates (paper VI) was consistent with the results of studies on liver cytosolic enzymes (98,99). The aldehyde dehydrogenase of human erythrocytes shares several other characteristics with the liver cytosolic enzyme (100,47): a high K_m for aldehydes, NAD^+ -dependent reactions, and disulfiram inhibition. The difference in AldDH activity between flushers and non-flushers is probably due to two types of affinity binding sites (99). Since the elimination in the first phase was almost identical for both flushers and non-flushers, it is unlikely that the difference in AldDH activity was due to a difference in the enzyme concentration. However, biphasic kinetics may have other causes such as the presence of different isoenzymes or enzyme co-operative behaviour (101).

Hillson and Hockaday (102), commenting on our paper VI, queried whether it was the 30 min incubation, the substrate concentration of 25 $\mu\text{mol/l}$ during phase 2, or a difference between the sexes that was the critical factor determining the results. In papers VI and VII, however, no difference in AldDH activity was found between females and males, and the substrate concentration after 30 min differed between the two studies: about 25 $\mu\text{mol/l}$ in paper VI, and 60 $\mu\text{mol/l}$ in paper VII. Thus, it seems likely that the 30 min incubation is the crucial factor, CPAF being always well established within 30 min (40).

Pyke's group (49) stated that CPAF may not be due to a variation of AldDH since the oriental type of AldDH has not been found in Occidentals (16). Liver biopsy specimens did not show atypical enzyme in flushers. However, they reported CPAF positive subjects to have higher blood acetaldehyde concentrations during disulfiram (Antabuse) alcohol flushing than CPAF negative subjects, and suggested a difference in AldDH activity between CPAF positive and CPAF negative individuals after exposure to enzyme inhibitors (55). Since, in addition, the basal disappearance rate of acetaldehyde in erythrocyte homogenates was slower in CPAF positive subjects than in

CPAF negatives (paper VI), Pyke's group (49) suggested AldDH in flushers to be more sensitive to the inhibitory effect of CP. Consistent with Pyke's proposal, Towell et al (97) reported differences in individual susceptibility to disulfiram AldDH inhibition. Hillson et al (103) demonstrated a relationship between AldDH activity and intensity of facial flushing after ethanol. In 4 of 6 probands the AldDH activity was reduced after 500 mg of CP, as compared with no reduction after placebo tablets. In the present study, AldDH activity was significantly inhibited by CP, but with great variation from one individual to another.

Prostaglandins

Strakosch et al (104) have shown aspirin to block CPAF in a majority of type 2 diabetic patients. In agreement with this finding, Barnett et al (51) found that CPAF was blocked by indomethacin in diabetics who were free from vascular complications (retinopathy and large-vessel disease) but not in those with such complications. Furthermore, twin studies suggested that the blockage had a genetic basis. In paper I, we reported naproxene to block CPAF. Aspirin, indomethacin and naproxene are prostaglandin synthesis inhibitors, acting on the cyclo-oxygenase. The results reported by Barnett et al also suggested prostaglandins to be involved in the pathogenesis of vascular complications in diabetes. Strakosch et al (105) reported an increase in prostacyclin concentrations during positive CPAF reactions, and that both the prostacyclin increase and the CPAF could be inhibited by either aspirin or indomethacin. Barnett et al (106) reported an increase in thromboxane during CPAF in diabetics whose CPAF reaction could be blocked by indomethacin, and that the thromboxane increase was also abolished by prior administration of the indomethacin. They found no significant increase in prostacyclin. Neither CPAF negative diabetics, nor those CPAF positive whose reaction was not blocked by indomethacin, showed any increase in prostanoid concentrations.

Horrobin and Manku (107) suggested blockage of the CPAF reaction by inhibitors of cyclo-oxygenase to be mediated by a peripheral, rather than a central mechanism. Their reasoning was that alcohol stimulates the production of prostaglandin E_1 from human platelets, the aspirin dose used was enough to inhibit platelet prostaglandin

synthesis but not brain or vessel-wall synthesis, and prostaglandin E_1 infusions produce facial flushing. Blockage of opiate receptors by naloxone will reduce the sensitivity of platelets to alcohol-induced activation of prostaglandin synthesis (108).

Endogenous opiates and tetrahydroisoquinolines

Leslie et al (41) reported CPAF to be blocked by the opiate antagonist naloxone and to be reproducible in response to an enkephalin analogue with opiate-like activity (DAMME). They suggested CPAF to be due to heightened sensitivity to endogenous opiates. Since substances with opiate-like activity affect carbohydrate metabolism and insulin secretion, this opiate sensitivity was suggested to give rise to type 2 diabetes associated with CPAF. Wheezing associated with CPAF could be prevented by naloxone, indicating asthma induced by CP and alcohol to be mediated by endogenous peptides with opiate-like activity (109). Hershon et al (110), studying the β -cell response to intravenous glucose in CPAF positive and CPAF negative type 2 diabetics, found no difference in insulin secretion before or after naloxone infusion between the two groups. At variance with this, however, Mason and Heber found naloxone to decrease the insulin secretion after oral glucose in CPAF positive diabetics, but not in CPAF negative diabetics (111), although Barnett et al were unable to confirm their findings (112). Thus, the suggestion that increased sensitivity to endogenous opiates may be involved in the pathogenesis of hyperglycemia in CPAF positive type 2 diabetic patients (41) has remained unconfirmed. Phillips and Lightman (108) suggested the effect of naloxone in CPAF to be due to perturbation of the opiate stimulatory effect on prostaglandin biosynthesis.

Medback et al (113) reported an increase in met-enkephalin concentrations after CP, which was even greater after both CP and alcohol together. Since neither the β -endorphin concentration nor the skin temperature changed significantly during the CPAF test, they suggested a lack of sensitivity to the action of met-enkephalin to explain the failure of CPAF negative individuals to flush. The increase in met-enkephalin concentrations remained unchanged when CPAF was blocked by naloxone (49). The increase in circulating met-enkephalin may be an effect of a direct action of alcohol on the gut. There was no increase in met-enkephalin concentrations after

intravenous alcohol despite the occurrence of the CPAF reaction (114).

Acetaldehyde reacts with naturally-occurring opioid peptides, enkephalins and β -endorphin. The stable acetaldehyde opioid adduct possesses less opiate activity than does the unchanged peptid (115). Acetaldehyde also reacts with dopamine to form salsolinol, and with dopaldehyde to form tetrahydropapaveroline. Salsolinol and tetrahydropapaveroline are tetrahydroisoquinoline alkaloids, derived from L-dopa metabolism (116). These alkaloids bind to opiate receptors in the rat brain. The potency of their opiate-like effects is comparable to that of the enkephalins and they are blocked by naloxone (117). Pyke's group reported preliminary data suggesting increased formation of tetrahydroisoquinolines in CPAF and that the increase was abolished when the flush was blocked (49).

Other possible mediators of CPAF

Increased blood concentrations and urinary excretion of epinephrine and norepinephrine have been reported in alcohol flushers after drinking (14,18). The symptoms and signs of alcohol flushing were considered to result from the sympathomimetic effects of acetaldehyde (14). Kupari et al (118) reported acetaldehyde-induced catecholamine release with peripheral vasodilatation, increased heart rate and decreased diastolic blood pressure in Orientals with genetically defective acetaldehyde oxidation. Strakosch et al (105) found increased blood norepinephrine concentrations in CPAF positive diabetics on CP therapy, as compared with CPAF negative diabetics who were not on CP. Significantly higher increases in heart rate after alcohol in CPAF positive subjects than in CPAF negatives have been reported (44, 64).

Since acetaldehyde competitively inhibits AldDH and the oxidation of ethanol reverses the NAD^+/NADH ratio, the favored pathway of serotonin metabolism is altered, resulting in a decrease of 5-hydroxyindoleacetic acid (5-HIAA) and an increase of 5-hydroxytryptophol (119). Patients with serotonin-producing tumours flush after drinking alcohol. Blood serotonin concentrations were increased in ethanol-sensitive subjects of Oriental descent (18). Metergoline, an anti-serotonin agent, however, did not affect CPAF (57).

Strakosch et al were unable to block CPAF with two anti-histamine drugs (104). Köbberling and Weber pointed out that the flush was abolished by a third anti-histamine drug (17). Thompson et al reported increased blood histamine concentrations during alcohol flushing in Orientals (18). Bromocriptine, a dopamine agonist did not affect CPAF (57). In a pilot study, we were also able to exclude changes in circulating concentrations of kinins as the active agents producing CPAF (120).

Soler and Motel reported CPAF in a patient started on CP treatment for diabetes insipidus, and who subsequently also developed diabetes mellitus (121). Among the sulfonylureas, chlorpropamide is the only effective potentiator of vasopressin. CP augments the effect of vasopressin on urinary excretion of prostaglandin E_2 , and possibly alters vascular response to vasopressin (122).

CLINICAL STUDIES (VII)

CPAF and AldDH activity in relation to prevalence of late diabetic complications

There was a weak, but significant ($p < 0.05$), relationship between facial flushing during CPAF tests and lack of late diabetic complications: 11 (50%) of 22 subjects flushed in the FVD (free from vascular disease) group, compared with 4 (17%) of 23 subjects in the LVD (large-vessel disease) group (paper VII). After correction for alcohol flush (true CPAF being reduced to 9 (41%) in the FVD group), the difference between the two groups did not reach statistical significance. The mean half-life ($T_{1/2}$) of acetaldehyde elimination (30-60 min) in erythrocyte homogenates was significantly shorter ($p < 0.005$) in the LVD group ($n=26$) than in the FVD group ($n=26$): 100 ± 11 min and 202 ± 28 min, respectively (Fig 1 in paper VII).

The mean half-life of acetaldehyde in 28 non-diabetic, non-alcoholic, healthy controls was 180 ± 21 min. Having two or more affirmative answers on the Mm-MAST questionnaire (39), three non-diabetic male controls had been excluded. Interestingly, in two of these excluded controls AldDH activity was extremely low, and the half-life was 521 in one and 629 min in the other. In 6 controls (21 %) the half-life was less than 100 min and in 9 (32 %) controls it was more than 202 min. AldDH activity in the control group was signifi-

cantly lower than in the LVD group ($p < 0.005$), but did not differ significantly from that of the FVD group.

The AldDH activity of the diabetics correlated to the total complication score ($p < 0.005$), macro-angiopathy score ($p < 0.05$), and peripheral neuropathy score ($p < 0.05$) (Fig 6, paper VII). These results were unaffected by anti-diabetic treatment, blood glucose control, alcohol consumption or by recognized risk factors of angiopathy such as blood pressure, hyperlipidemia or smoking.

Discussion

Micro-angiopathy

In their first paper on CPAF and late diabetic complications Leslie, Barnett and Pyke (4) demonstrated retinopathy to be commoner and more severe in type 2 diabetics who were CPAF negative than in those who were CPAF positive. The CPAF negative patients had early signs of retinopathy. Within 5 years after the diagnosis of diabetes, about 25% of the CPAF negatives had retinopathy, as compared

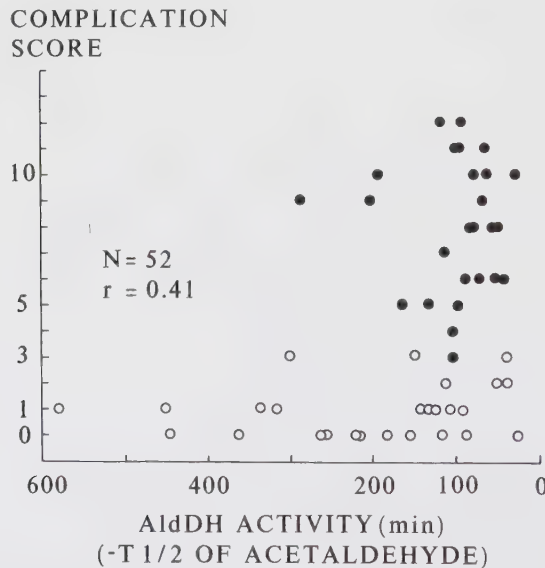


Fig.6. Aldehyde dehydrogenase activity (= - half-life of acetaldehyde between 30-60 min) compared to a complication score in 26 type 2 diabetics with large-vessel disease (●) and 26 diabetics free from clinical vascular disease (○).

with less than 10% of CPAF positives. Furthermore, the difference between the two groups increased strikingly with the duration of disease. In neither of the two CPAF groups, was diabetes in the family associated with freedom from retinopathy. Leslie, Barnett and Pyke (123) also reported micro-angiopathic complications among type 1 diabetic patients to be less common in CPAF positives than in CPAF negatives.

Radder et al (59) confirmed that retinopathy was less common in type 2 diabetic patients with CPAF than in those without the CPAF reaction. In several studies (45,52,79,124) a tendency was reported for CPAF positive diabetic patients to be less prone to develop retinopathy than CPAF negative patients. However, in other studies (71,72,125) no correlation whatsoever was found between retinopathy and CPAF. One study, while showing no difference in background retinopathy to exist between the two CPAF groups, reported severe loss of visual acuity to be confined to the CPAF negative diabetics (67).

In type 2 diabetics, CPAF has been shown to be associated with a relative freedom from nephropathy (persistent proteinuria) (126). Other investigators (45,124) have found a tendency for CPAF positive diabetics to have less signs of nephropathy than CPAF negative diabetics.

Macro-angiopathy

In 1980 both Pyke's group (5) and our own group (6) were able to show macro-angiopathy (intermittent claudication, absent foot pulses and/or ischemic heart disease) to be less common among CPAF positive diabetics than among CPAF negative diabetics, and a similar tendency was found in the present study. However, determining the basal erythrocyte AldDH activity, in turn partially responsible for the CPAF reaction, might be a preferable method. Large-vessel disease was significantly more common in patients with higher AldDH activity than in those in whom it was lower. Some reports (52,67) confirm the finding that CPAF positive diabetics are less prone to develop macro-angiopathic complications than CPAF negative patients. In other studies (79, 124, 127), no difference between CPAF positive and CPAF negative patients regarding macro-angiopathy was found, while one paper (45) reported a tendency for CPAF positive diabetics

to have a lower prevalence of macro-angiopathic complications than CPAF negative diabetics.

Peripheral neuropathy

Signs of peripheral neuropathy (heightened vibration sense threshold and loss of Achilles tendon reflexes) among diabetics were found more often in CPAF negatives than in CPAF positives (6). Although Boulton et al (128) were unable to confirm this finding, Kardoyannis et al (124) found CPAF positive diabetics to have a tendency for fewer neuropathic manifestations than CPAF negative diabetic patients. In the present study, AldDH activity was significantly higher in diabetics with more advanced peripheral neuropathy than it was both in non-diabetics or in diabetics with less advanced neuropathy or none at all. However, the patients in study VII were chosen on the ground of symptoms and signs of large-vessel disease, and those with angiopathy invariably had fewer manifestations of neuropathy than matched probands without angiopathy.

GENERAL DISCUSSION

The suggestion that the chlorpropamide alcohol flush (CPAF) phenomenon is genetically determined and associated with relative freedom from angiopathy (3,4,5) provided the basis for the present investigations.

The thesis especially deals with three aspects of CPAF: its objective assessment, its underlying mechanisms, and its possible relationship to late diabetic complications. During the course of these studies, it became clear that aldehyde dehydrogenase (AldDH) was implicated in CPAF. The activity of this enzyme might be of importance for the metabolism of other aldehydes than acetaldehyde and thus possibly be involved in the pathogenesis of angiopathy.

Does CPAF in fact exist? The phenomenon is clearly distinct from that of the alcohol flush since it comes on from the start of chlorpropamide treatment, persists for the duration of therapy, and invariably ceases within a week of therapy being terminated. The prevalence of CPAF reported in the early literature was 25-34% (25, 29,30). With the single-dose CPAF test, reports of the frequency of the flush in type 2 diabetics varied as widely as 17% and 52% (Table III). Although Pyke's group reported CPAF to be more common in type 2 than in type 1 diabetics and non-diabetic subjects (3), other investigators found no difference in CPAF frequency between the three groups (68). It is now clear that the pretreatment CP dose is critical for the outcome for the test (paper IV, 53,54,85). It has been suggested that the positive correlation between the plasma CP concentration and intensity of the flush is no longer evident at CP concentrations above 40 mg/l, and that a CP dose sufficient to give rise to a plasma concentration of 40 mg/l or above should be capable of detecting all potential flushers (48). However, neither the data presented in paper V, nor those published by Hillson et al (53), support the actual existence of any such "threshold". Body-weight-matching of the CP dose seems to be important: plasma CP concentrations correlate inversely to body weight. This probably accounts for the sex difference found in the prevalence of CPAF, more females than males being flushers (paper V, 84).

Is it possible to measure the CPAF reaction? Assessment by subject and observer tends to be imprecise, while facial skin temperature increase has been reported to be unreliable by some investigators (59,60), and to be precise and objective by others (52,57). In the present study (papers II-III) there was good agreement between observer assessment and measured temperature increase, and any influence of basal facial skin temperature on the increment was insignificant. However, other investigators urge that the starting skin temperature must be taken into consideration (43,48). In paper I, the determination of acetaldehyde concentrations was put forward as a new approach to the objective assessment of CPAF, and the distinction between flushers and non-flushers was unequivocal. In a larger material the difference between the two groups was highly significant, albeit with some overlap (paper II). The sensitivity and specificity of the skin temperature and plasma acetaldehyde methods was further enhanced when the methods were used in combination, as in CPAF Criteria and CPAF Index (papers II, V). Thus, with these methods objective study of CPAF is considered feasible.

What are the biochemical events following exposure to CP and alcohol? As already mentioned, the size of the CP dose is critical. It is clear that CP inhibits the AldDH activity, creating an accumulation of acetaldehyde (paper I, 56,64). However, the CP dose is not the sole determinant of the acetaldehyde increase. Basal AldDH activity was found to be another important factor determining the reaction to CPAF challenge tests (paper VI, 103). The size of the alcohol dose may also be important (64), although, this has not been generally accepted (48).

An increase in acetaldehyde seems to be the first metabolic step in CPAF (63). How do acetaldehyde concentrations induce facial flushing? Several other mediators of CPAF have been put forward; for instance, there is evidence that the flush might be mediated by a sensitivity to increased met-enkephalin concentrations (113). Again, the activity of endogenous opioids may be altered by acetaldehyde (115), which suggests a link between the increase in acetaldehyde and the possible involvement of opioids in the mediation of the CPAF reaction. Another possible link between an opiate effect and acetaldehyde is the formation of tetrahydroisoquinolines, compounds

which have an opiate-like activity and are formed as a result of a reaction between endogenous amines and acetaldehyde. It has been suggested that these compounds are increasingly formed during CPAF (49). Acetaldehyde accumulation induces a catecholamine release (14). The β -adrenergic blocking agent, propranolol, has been reported to modulate flush response to alcohol (61). Prostaglandin synthetase inhibitors may block CPAF (paper I, 51,104), and an increase in prostaglandin concentrations has been reported during CPAF (18, 105,106).

Alcohol-induced activation of opiate receptors is capable of increasing the synthesis of the vasodilator prostaglandin E_1 from human platelets (107). This effect of alcohol might be enhanced by acetaldehyde accumulation, in turn altering the activity of opioids and accelerating the formation of tetrahydroisoquinolines with their opiate-like activity. This hypothesis may explain why some subjects flush without any increase in their acetaldehyde concentrations, so-called alcohol flushing. In such subjects, enhancement of prostaglandin biosynthesis by acetaldehyde is redundant and the alcohol-effect alone suffices to produce facial flushing. Obviously, of course, simple alcohol flushers may also manifest CPAF (paper II, 48).

Do any metabolic differences exist between flushers and non-flushers?

Since reports on insulin and C-peptide pattern, basal and after intravenous or oral glucose load, have been conflicting (58, 71,110,111,112), it is not possible to suggest a relationship between CPAF pattern and insulin secretion. Moreover, the suggested relationship between endogenous opiates, CPAF and insulin secretion in type 2 diabetes (41) has not been confirmed in metabolic studies (110,112). In one study, however, naloxone decreased insulin secretion in response to oral glucose in CPAF positive diabetics but not in CPAF negative diabetics (111). Although several investigators have been unable to detect any differences in lipid variables between flushers and non-flushers (paper VII, 67,124), Karamanos et al (44) found higher concentrations of high density lipoproteins (HDL) and lower concentrations of low density lipoproteins (LDL) in flushers than in non-flushers. Barnett et al (112) reported increased

concentrations of serum triglycerides in CPAF positive diabetics with retinopathy, whereas both CPAF positive patients without retinopathy and CPAF negative patients had near normal serum lipids.

Could CPAF be used to predict future diabetes in non-diabetic subjects? CPAF was reported to be a dominantly inherited trait associated with diabetes (3), and was also suggested to be common in diabetics with diabetic relatives (4). Later, several studies have failed to show either that CPAF is inherited, or that it is associated with diabetes in the family (paper II, 50,59,67,76). It seems that the inheritance of CPAF is restricted to certain families (48).

Could CPAF be used as a tool in predicting future diabetic complications? Several studies have shown CPAF positive type 2 diabetics to have less retinopathy, nephropathy, large-vessel disease and peripheral neuropathy than CPAF negative diabetics (4,5,6,59,67,126). Other studies found no significant differences in the prevalence of diabetic complications between flushers and non-flushers (71,72,79,124,125,127). Since the size of the CP premedication dose is critical for the outcome of the test, it is necessary to establish whether freedom from complications is associated with low-dose-CPAF ("easy flushers") or high-dose-CPAF ("potential flushers"), and whether inability to flush ("flush incapables") is associated with diabetic complications.

There was a possible relationship between CPAF and diabetic complications, as well as between erythrocyte AldDH activity and CPAF (paper VI). Could the basal AldDH activity, rather than the biochemical events after CP and alcohol addition, be the more likely variable associated with late diabetic complications? The results of paper VII strongly support this hypothesis. High AldDH activity was significantly more in evidence in vascular disease than were any of the recognized risk factors of angiopathy.

It is not clear whether the AldDH activity is a primary factor involved in the pathogenesis of vascular disease or merely a secondary phenomenon, though some arguments favor its being a primary factor. These arguments include studies on hepatic cytosolic AldDH activity and alcoholism (129), genetic studies on the inheritance of CPAF (3), lack of AldDH isozyme in Japanese (16), liver function tests being without association to AldDH activity (130), studies on

alcohol consumption, vascular disease (131,132) and decreased AldDH activity (11), and the finding of two distinct categories among both non-diabetics and diabetics free from vascular disease - those with higher AldDH activity and those with lower (further details are to be found in paper VII). If the AldDH activity is a primary factor in vascular disease, it may serve as a marker of increased risk of late diabetic complications. Manipulation of the enzyme activity is already possible with either disulfiram (133), chlorpropamide (9, 134) or alcohol (11). If the AldDH activity is merely a secondary phenomenon, it may nonetheless help us to understand more about the pathogenesis of angiopathy.

It is obvious that AldDH, its biochemical actions and its possible role in the pathogenesis of diabetic complications, requires further study. Studies on AldDH activity in non-diabetic patients with angiopathy, and in type 1 diabetes with micro-angiopathy are needed. Furthermore, re-evaluation of the diabetics free from clinical angiopathy but with high AldDH activity in paper VII is required to establish whether, in contrast to those with low AldDH activity, they are liable to develop diabetic complications in the future.

SUMMARY AND CONCLUSIONS

The thesis summarizes investigations carried out on type 2 diabetic patients (papers I-VII). The chlorpropamide alcohol flush (CPAF) was studied from methodological, pharmacological, biochemical and clinical aspects. The studies were carried out with special emphasis on skin temperature methods, plasma chlorpropamide and acetaldehyde concentrations, aldehyde dehydrogenase activity, and possible relationship between CPAF and late diabetic complications.

The following observations of interest were made:

1. The chlorpropamide alcohol flush is a common phenomenon in type 2 diabetic patients.
2. In the majority of type 2 diabetics, the occurrence of CPAF is independent of any first-degree family history of diabetes.
3. CPAF is accompanied by an increase in plasma acetaldehyde concentrations, which can be used as an objective marker of the phenomenon.
4. CPAF can be studied objectively using facial skin temperature methods. Specificity and sensitivity are nearly 100% when temperature and acetaldehyde determination methods are used in combination.
5. Chlorpropamide-induced inhibition of aldehyde dehydrogenase with subsequent increase in acetaldehyde concentrations constitutes the first metabolic step of CPAF. Other mediators, such as endogenous opiates, prostaglandins, tetrahydroisoquinolines and catecholamines, may be involved in secondary metabolic steps leading to facial vasodilatation.
6. The common alcohol flush and CPAF are distinct phenomena, though both may be found in the same individuals. Increase in blood acetaldehyde concentrations, typical of CPAF, is not found in

alcohol flushing. However, the two phenomena may share other flush mechanisms.

7. The frequency of CPAF, as well as the individual intensity of the flush, correlates to the size of the CP premedication dose.
8. Lower body weight in females than in males is associated with higher plasma CP concentrations and higher prevalence of CPAF.
9. Plasma chlorpropamide is not the sole determinant of the subsequent increase in blood acetaldehyde concentration. The activity of aldehyde dehydrogenase (AldDH) is critical and alcohol concentrations may also be important.
10. Basal erythrocyte AldDH activity is significantly lower in flushers than in non-flushers, is reduced by CP, and is associated with a greater increase in the blood acetaldehyde concentration during CPAF.
11. CPAF and basal AldDH activity are correlated to presence of late complications in type 2 diabetic patients. High AldDH activity is more in evidence in vascular disease than are several of the recognized risk factors of angiopathy.

Thus, a clinical observation in a purely social context of a mother and her daughters, all on chlorpropamide therapy, manifesting a facial flush after drinking sherry (135), has led to an extensive series of studies of the phenomenon. Investigations were made on the relationship between CPAF and type 2 diabetes mellitus, particular with reference to diabetic complications. A period with very promising results was succeeded by a period with conflicting results and doubt as to the possible implications of the phenomenon. The results from the studies presented in this thesis are an indication of the importance of CPAF in research on diabetes mellitus. Studies on aldehyde dehydrogenase may prove to be the key to understanding of the relationship between CPAF and late complications in diabetes mellitus.

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Rapid Publications

Increase of Plasma Acetaldehyde

An Objective Indicator of the Chlorpropamide Alcohol Flush

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SUMMARY

Chlorpropamide alcohol flushing (CPAF) in non-insulin-dependent diabetics (NIDDs) has been reported to be associated with a lower tendency to develop late complications. The flush was thought to be mediated by enkephalins and prostaglandins. Early studies could not correlate CPAF to increased levels of acetaldehyde in blood and the flush was not regarded as an antabuse-like reaction.

In this study, the increase of plasma acetaldehyde during the flush in 13 CPAF positive diabetics was significantly ($P < 0.005$) higher than in 13 CPAF negative diabetics during a CPAF challenge test. The increase of plasma acetaldehyde was reduced to the level of CPAF negative diabetics in three CPAF positive diabetics when they were exposed to alcohol without premedication with chlorpropamide and they did not flush. The normal breakdown of ethanol to acetic acid via acetaldehyde appears to be inhibited by chlorpropamide in the flushers. Acetaldehyde measurement is an objective method to study the chlorpropamide alcohol flush and it appears superior to the measurement of skin temperature. DIABETES 30:788-791, September 1981.

A well known side effect of chlorpropamide medication in some diabetics is a facial flush after drinking alcohol, even in small amounts.¹ This chlorpropamide alcohol flush (CPAF) is found in many patients with non-insulin-dependent diabetes mellitus (NIDD) and it has been suggested that it is an autosomal dominant inherited trait.² The CPAF positive diabetics are reported to have a lower prevalence of diabetic retinopathy³ as well as macroangiopathy^{4,5} and peripheral neuropathy⁵ than the CPAF negative diabetics.

In many patients, however, the presence or absence of flush during a CPAF challenge test is difficult to judge. This has made many investigators uncertain about the prevalence and specificity of the flush. An endogenous opiate, enkephalin, can induce a similar flush and CPAF can be blocked by naloxone.⁶ Inhibitors of cyclooxygenase, such as aspirin^{7,8} and indomethacin,⁹ reduce the flush in CPAF positive diabetics who are free of vascular complications, suggesting that prostaglandins are also involved.

CPAF was not believed to be an antabuse-like reaction because an investigation by FitzGerald et al.¹⁰ did not find any correlation between blood levels of acetaldehyde and the flush. It was suggested that an antabuse-like reaction should appear in all patients during an alcohol challenge test and would not be an inherited trait.²

Ethanol is metabolized via acetaldehyde to acetic acid, and the enzymes responsible for these reactions are alcohol dehydrogenase and aldehyde dehydrogenase, respectively. Since chlorpropamide most likely inhibits the aldehyde dehydrogenase,¹¹ we wanted to study the levels of acetaldehyde during the chlorpropamide alcohol flush.

PATIENTS AND METHODS

Following the method of previous studies, we selected 13 clearly CPAF positive and 13 clearly CPAF negative patients with NIDD. Eight of the flushers and four of the non-flushers were women. The mean age was 69 (59-82) yr in the flushing group and 60 (33-76) yr in the nonflushing group. The mean duration of diabetes after diagnosis was 8.8 (2-22) yr and 4.7 (1-12) yr, respectively. Four of the CPAF positive diabetics were on diet only, seven were on maintenance therapy with chlorpropamide (two on 125, two on 250, and three on 375 mg daily) and two were on other sulphonylurea drugs. Four of the CPAF negative diabetics were on diet only, three were on chlorpropamide (one on 125 and two on 250 mg daily), and six were on other sulphonylurea drugs.

CPAF challenge test. Sixteen of the patients had a standard dose of 250 mg chlorpropamide 12 h before the CPAF test. Three patients had 125 mg, four patients had 250 mg,

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and three patients had 375 mg in the morning because they were on maintenance therapy with those dosages.

The patients were studied in a room with constant temperature and the skin temperature was measured every 5 min 2 cm below the lateral canthus of the left eye during the test. A sensitive temperature probe from Electrolaboratory (Copenhagen) was used. When the skin temperature seemed to remain constant (after 20–30 min), the patients had 8 g of pure alcohol in fruit juice. After 30 min, the appearance or absence of flush was judged by us and by the patients themselves.

Plasma acetaldehyde and ethanol. Samples for plasma acetaldehyde and ethanol were taken before and 25 min after the alcohol ingestion. Plasma levels of ethanol and acetaldehyde were determined by head space gas chromatography. Acetaldehyde was derivatized with semicarbazide by a modification of the method described by Stowell.¹² Blood samples were collected in 10-ml vacuum tubes containing 3 ml buffered semicarbazide solution, prepared fresh for every trial to avoid artifactual formation of acetaldehyde (Lindros, personal communication). Blood cells were separated by centrifugation in graded tubes, and 2 ml of the diluted plasma samples thus obtained was transferred to pre-cooled 25-ml glass vials. Seventy percent perchloric acid (0.1 ml) was added to hydrolyze the acetaldehyde semicarbazone. Tert-Butanol was used as internal standard. The glass vials were sealed with a rubber membrane and incubated at a temperature of 65°C for 20 min. Five milliliters of the head space was injected in a Varian 2400 gas chromatograph equipped with a flame ionization detector. A Porapak Q column was used, maintained at 110°C.

Alcohol challenge test. In three other clearly CPAF positive diabetics, we performed an alcohol challenge test. This was done in the same way as a CPAF challenge test, but without premedication with chlorpropamide. We waited at least 10 days after the CPAF challenge test before this test was performed. The Student's *t* test was used for statistical analysis.

RESULTS

As expected, the 13 previously known CPAF positive patients flushed, while the 13 CPAF negative patients did not. A very intense flush was seen in three women, while two flushing men had only a light flush. Of the CPAF negative patients, no one claimed to feel facial warmth and no facial redness was observed.

Skin temperature. The two groups (CPAF positive and negative patients) had a starting skin temperature of $32.6 \pm 0.3^\circ\text{C}$ and $33.4 \pm 0.4^\circ\text{C}$ (mean $\pm$ SEM, N.S.), respectively. Thirty minutes after ethanol administration, the increase in skin temperature of the flushers was $2.2 \pm 0.4^\circ\text{C}$ (mean $\pm$ SEM), while the nonflushing patients had an increase of only $0.6 \pm 0.2^\circ\text{C}$ (mean $\pm$ SEM, $P < 0.001$). Two of the clearly CPAF patients had an increase less than 1°C , while three of the clearly CPAF negative patients had an increase in facial temperature of 1°C or more (Figure 1).

Plasma ethanol. Only minimal concentrations (mean $2.2 \text{ mmol/L} = 1/10 \text{ promille}$) were found in all patients tested. The CPAF positive patients had a plasma ethanol level at $2.8 \pm 0.7 \text{ mmol/L}$ and the CPAF negative patients had a level at $1.7 \pm 0.2 \text{ mmol/L}$ (mean $\pm$ SEM, N.S.). Two flushing

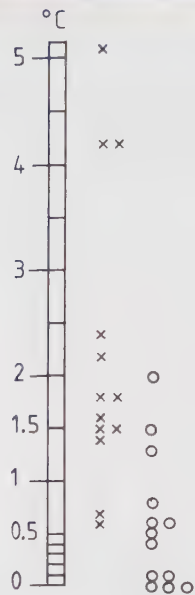


FIGURE 1. Increase in skin temperature 30 min after alcohol ingestion during a CPAF challenge test in 26 diabetics. Flush (X). No flush (O).

patients had somewhat higher levels (8 and 5 mmol/L, respectively).

Plasma acetaldehyde. Baseline levels of plasma acetaldehyde were below or at the detection level. Twenty-five minutes after the ethanol administration, the assays of plasma acetaldehyde showed an increase of $15.0 \pm 4.0 \mu\text{mol/L}$ in the CPAF positive but only an increase of $2.1 \pm 0.2 \mu\text{mol/L}$ (mean $\pm$ SEM) in the CPAF negative patients ($P < 0.005$). This increase was greater in all of the flushers than in any of the nonflushers. In 8 of the 13 flushers, there was a considerable increase (Figure 2).

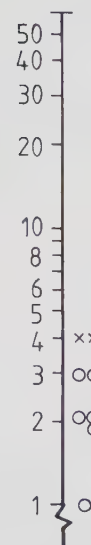
Alcohol challenge test. The mean skin temperature increase was reduced from 3.7°C (range 3.0 – 5.1°C) at the CPAF challenge tests to 1.2°C (range 0.7 – 1.7°C) at the alcohol challenge tests.

Plasma ethanol levels were the same in all three patients at both challenge tests but the mean increase of plasma acetaldehyde was reduced from $13.3 \mu\text{mol/L}$ (range 6–26) to $3.7 \mu\text{mol/L}$ (range 3–4).

DISCUSSION

Plasma acetaldehyde is elevated during the chlorpropamide alcohol flush and, as acetaldehyde is the first product in the metabolism of ethanol, this would be the first metabolic step of the flush.¹³

There are many problems with skin temperature registrations. Two of the 13 clearly flushing patients had an increase less than 1°C during the flush. In one patient, the explanation was that the flush was localized to the nose and did not reach the point where the skin thermometer probe



In 1962, FitzGerald et al.¹⁰ investigated four patients by measuring blood acetaldehyde during a chlorpropamide alcohol flush. They could not find any differences following ethanol ingestion with or without chlorpropamide treatment. Acetaldehyde was determined on whole blood samples by Stolz's method after distillation into bisulphite, and ethanol

In conclusion, we have demonstrated that the chloropropamide alcohol flush is correlated to a rise of plasma acetaldehyde levels. The acetaldehyde measurement appears superior to skin temperature measurement in providing an objective method to study CPAF.

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The chlorpropamide alcohol flush test in diabetes mellitus: methods for objective evaluation

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In order to study the objective value of the chlorpropamide alcohol flush (CPAF) the facial skin temperature and plasma acetaldehyde methods were compared to the visible response (flush/no flush) on standardized CPAF and alcohol challenge tests in 137 type 2 diabetics. Three criteria of CPAF are defined. A visible facial flush was noted in 53% of the diabetics. An increase in facial skin temperature of at least 1.0°C was found in 90% of the subjects with a visible facial flush (flushers), but in only 14% of non-flushers. An increase in plasma acetaldehyde of at least 4 $\mu\text{mol/l}$ was found in 86% of the flushers and in only 15% of non-flushers. Using these criteria to study CPAF all flushers satisfied at least two and 78% fulfilled all three criteria, while no non-flusher fulfilled more than one and 74% satisfied no CPAF criteria. However, with the alcohol test 5% could be identified as alcohol flushers having a falsely positive CPAF-test. In conclusion, it was possible to evaluate the CPAF test objectively with the facial skin temperature and plasma acetaldehyde methods.

Key-words: acetaldehyde; chlorpropamide alcohol flush; diabetes mellitus; facial flush; skin temperature

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Alcohol intolerance as a side effect of chlorpropamide (CP) medication has been known for many years [3]. Leslie & Pyke reported the chlorpropamide alcohol flush (CPAF) to be an autosomally dominant inherited trait in non-insulin dependent (type 2) diabetes [14]. Lower prevalences of diabetic retinopathy [13], large-vessel disease [2, 6], peripheral neuropathy [6] and diabetic nephropathy [1] were reported in CPAF-positive than in the CPAF-negative type 2 diabetics.

Objective methods to evaluate the CPAF test

have been inadequate. The facial skin temperature [15] showed a considerable overlap between flushers and non-flushers [18, 4] causing difficulties in clearly separating the two groups. Furthermore, many flushers also flushed on alcohol challenge tests without CP premedication, hence the specificity of the flush was questioned [12]. Increased levels of plasma acetaldehyde were found in flushers [9] as a result of non-competitive inhibition of aldehyde dehydrogenase by chlorpropamide [17]. Determination of the acetaldehyde level might be used as an objective indicator of a process related to the flush [10].

The purposes of this study were to compare

the elevations of facial skin temperature and plasma acetaldehyde to the visible response (flush/no flush) on standardized CPAF and alcohol tests, and evaluate a combination of these indicators in the classification of the outcome of the CPAF test.

SUBJECTS AND METHODS

From our out-patient clinic 137 type 2 (maturity-onset, non-insulin dependent) diabetics, 61 females and 76 males, were studied. The median age was 70 years (range 16–83 years) and the mean duration after diagnosis was 9 years (range 1–22 years). Thirty-one patients were on maintenance therapy with 125, 250 or 375 mg chlorpropamide daily and 47 patients were on other sulphonylurea drugs (mainly glibenclamide or glipizide). Seventeen patients were, after several years on sulphonylurea, on insulin treatment with 12 to 56 U daily. The remaining 42 patients were on diet only. We used 35 healthy males as controls.

The CPAF test and facial skin temperature

Standard doses of 250 mg (in all controls and in the 73 first tested patients) or 375 mg (in the 33 last tested patients) of chlorpropamide (Diabines, Pfizer) were taken 12 h before the test. No other drugs were allowed after the CP administration. Patients on maintenance CP therapy had their usual dose in the morning of the test day. All patients were fasting at least 2 h before the test. The patients were studied in a room with constant temperature. Skin temperature was measured 2 cm below the lateral canthus of the left eye every 5 min during the test. A sensitive temperature probe from Electrolaboratory, Copenhagen, was used. When the skin temperature seemed constant (usually after 20–30 min) the patients had 8 g of pure ethanol in fruit juice. After another 30 min the appearance or absence of flush was judged by one examiner who was the same during the whole study.

Plasma acetaldehyde

Blood samples (triplicates) were taken 25 min after the alcohol ingestion, the time of maximum flushing in most subjects [10]. The concentration

of acetaldehyde were, after hydrolysis with perchloric acid, determined in plasma by gas chromatography of head space samples [20, 10].

The three CPAF criteria

Criteria were chosen to separate the CPAF-positive from CPAF-negative subjects: the basis criterion or the *first CPAF criterion*, the visible facial flush [14], and two additional criteria. Since it has been reported that an increase in facial skin temperature of at least 1.0°C is seen in most flushers [11, 19] this was defined as the *second CPAF criterion*. Based on our earlier experience [10] an increase in the plasma acetaldehyde level of at least 4 µmol/l was defined as the *third CPAF criterion*.

Alcohol tests

In subjects positive to the single-dose CPAF test, tests were repeated more than 10 days later. This time only alcohol, was given. A positive alcohol test was defined as a visible flush and a temperature increase $\geq 1^\circ\text{C}$. An increase in plasma acetaldehyde could not be expected since no inhibitor of the aldehyde dehydrogenase was given [10].

Statistics

For statistics we used the chi-square test or Student's *t*-test after logarithmation of results with crooked distribution. All data are given as mean $\pm$ SEM.

RESULTS

In 73/137 (53%) of the CPAF tests the subjects were judged as flushers by the examiner. Among the patients on maintenance therapy with CP 28/31 (90%) were judged as flushers compared to 45/106 (42%) after the single-dose CPAF tests ($P < 0.001$). Flushing was not more common after single-dose premedication with 375 mg than after 250 mg CP. In diabetics on diet only flushing was unusual in males compared to females: 3 of 18 (17%) males v 14 of 24 (58%) females flushed ($P < 0.02$). This difference between the sexes was not seen in the other therapeutic subgroups (CP, other sulphonylureas, insulin). In 31 (42%) of the flushers and in 32 (50%) of the non-flushers a

TABLE I. Facial skin temperature (mean $\pm$ SEM) in 137 type 2 diabetics. A comparison between CPAF positive and CPAF negative diabetics, as well as between females and males, regarding starting facial skin temperature and increase in facial skin temperature, respectively

	Starting facial skin temperature t_0 °C			Increase in facial skin temperature ΔT_{30} °C		
	All subjects tested	Females	Males	All subjects tested	Females	Males
CPAF positive	32.1 $\pm$ 0.1	31.8 $\pm$ 0.2	32.5 $\pm$ 0.2§	1.9 $\pm$ 0.1	2.3 $\pm$ 0.2	1.6 $\pm$ 0.1§
CPAF negative	32.5 $\pm$ 0.2*	32.0 $\pm$ 0.3	32.8 $\pm$ 0.2‡	0.6 $\pm$ 0.0†	0.7 $\pm$ 0.1†	0.5 $\pm$ 0.1†
All subjects tested		31.9 $\pm$ 0.2	32.7 $\pm$ 0.1¶			

*CPAF positive compared to CPAF negative (NS).

†CPAF positive compared to CPAF negative ($P<0.001$). §CPAF positive: females compared to males ($P<0.005$).

‡CPAF negative: females compared to males ($P<0.05$).

¶All females compared to all males ($P<0.001$).

first degree family history was known (NS). In non-diabetic controls 5/35 (14%) had a visible flush. No hypoglycemic episode was reported.

Facial skin temperature

The mean temperature of the test room was 22.5°C during all tests. The mean starting facial skin temperature (mean t_0) was the same in flushers and non-flushers (Table I). However, females had a lower t_0 than males ($P<0.001$). The mean increase in facial skin temperature after 30 min (mean ΔT_{30}) was higher in the flushers than in the non-flushing subjects, 1.9 $\pm$ 0.1 and 0.6 $\pm$ 0°C, respectively ($P<0.001$) (Fig. 1). Mean ΔT_{30} was higher in female than in male flushers ($P<0.005$) (Table I).

The highest facial skin temperature measured was 36.2°C. The controls with a visible flush had a ΔT_{30} of 1.0–1.6°C, while the non-flushing controls had a ΔT_{30} of 0–0.8°C.

Plasma acetaldehyde

Baseline levels of plasma acetaldehyde were at the detection level or below. The mean Δ P-Acetaldehyde (mean increase in plasma acetaldehyde concentration 25 min after the alcohol ingestion) was 9.7 $\pm$ 1.4 μ mol/l in the flushers and 2.6 $\pm$ 0.2 μ mol/l in the non-flushers ($P<0.001$) (Fig. 2). Acetaldehyde could not be determined in the controls.

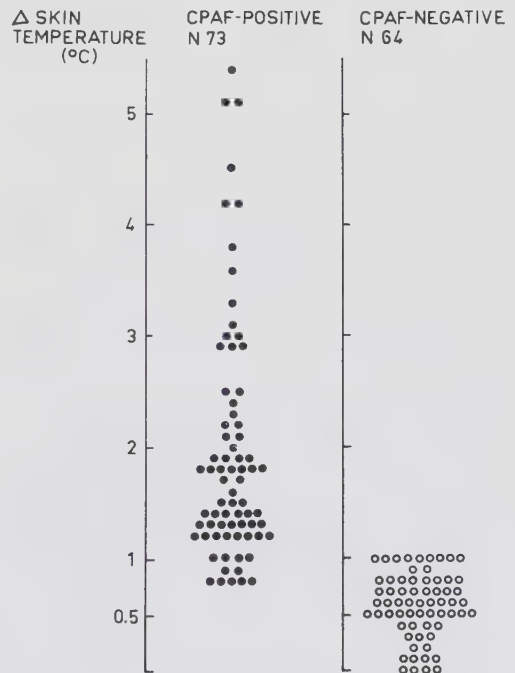


FIG. 1. Increase in facial skin temperature (30 min) in CPAF positive (●) and CPAF negative (○) subjects during CPAF tests. An increase of $\geq 1.0^\circ\text{C}$ represents the second CPAF criterion.

The CPAF criteria

Since the first CPAF criterion was the basis for the separation of the two groups all 73 flushers

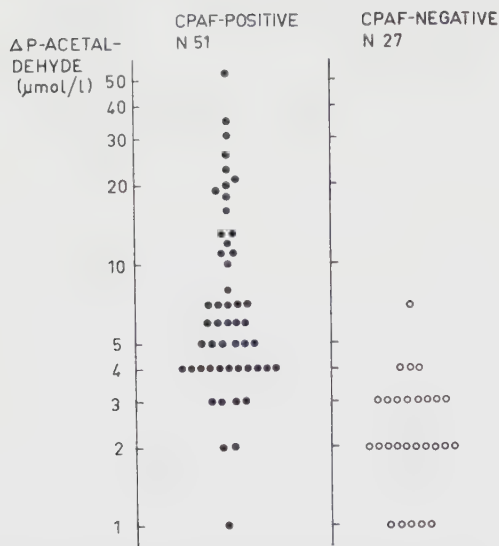


FIG. 2. Increase in plasma acetaldehyde levels (log scale) in CPAF positive (●) and CPAF negative (○) diabetics during CPAF tests. A level at $\geq 4 \mu\text{mol/l}$ represents the third CPAF criterion.

and no non-flusher fulfilled this criterion. The second CPAF criterion ($\Delta T_{30} \geq 1.0^\circ\text{C}$) was satisfied by 90% of the flushers and 14% of the non-flushers (Fig. 1), while 86% of the flushers and 15% of the non-flushers fulfilled the third CPAF criterion ($\Delta\text{P-Acetaldehyde} \geq 4 \mu\text{mol/l}$) (Fig. 2).

In 78% of the flushers all CPAF criteria were fulfilled, while in 22% two criteria were satisfied. In the non-flushers, however, 74% had all three criteria negative and 26% had only one additional criterion positive. There was no overlap between the groups.

Alcohol tests

Compared to the CPAF tests, mean ΔT_{30} , as well as mean $\Delta\text{P-Acetaldehyde}$, were lower during the alcohol tests in the 41 CPAF positive diabetics: $1.9 \pm 0.2^\circ\text{C}$ and $0.8 \pm 0.1^\circ\text{C}$ ($P < 0.001$), $7.4 \pm 1.3 \mu\text{mol/l}$ and $1.9 \pm 0.3 \mu\text{mol/l}$ ($P < 0.001$), respectively. In 14 (34%) of these flushers, four having satisfied first and second and 10 all three criteria during the CPAF test, there was a visible facial flush and a $\Delta T_{30} \geq 1.0^\circ\text{C}$ also during the alcohol test. How-

ever, in six the flush was less intense and the skin temperature increased less during the alcohol test than during the CPAF test. $\Delta\text{P-Acetaldehyde}$ did not exceed $4 \mu\text{mol/l}$ during any alcohol test and there was no difference between females and males.

DISCUSSION

A visible facial flush during a CPAF test (the basis CPAF criterion) could be confirmed in 90% by the method of increased facial skin temperature and in 86% by the method of increased plasma acetaldehyde levels. The absence of facial flush was confirmed with the same confidence with these two methods. Furthermore, in 26 flushers the results were confirmed by repeated tests.

Since all flushers also fulfilled both (78%), or one (22%), of the additional CPAF criteria they were classified as CPAF positive diabetics. Lacking all three CPAF criteria (74%), or only satisfying one additional criterion (26%), all non-flushers in this study consequently were classified as CPAF negative.

The fact that 34% of the CPAF-positive diabetics also experienced a flush during the alcohol test makes it necessary to discuss the relation between CPAF and the simple alcohol flush. We believe the CPAF and alcohol flush to be related phenomena. Firstly, the facial flush on alcohol challenge, with or without CP premedication, seemed identical (however less intense in some) to the observer and the subjects tested. Secondly, the two reactions could partly be mediated by the same mechanisms, possibly involving prostaglandins [21] and endogenous opiates [15]. It was suggested that alcohol and opiates stimulate the production of prostaglandin E [5] and recently acetaldehyde has been shown to form adducts with some endogenous opiates [16]. We therefore suggest that in most subjects elevated acetaldehyde levels are necessary to induce the mechanisms leading to a facial flush. However, some subjects are able to flush even without detectable acetaldehyde elevation during the alcohol test but, as an effect of CP-induced inhibition of aldehyde dehydrogenase [17], have elevated levels during the CPAF test. The various outcomes of CPAF and alcohol tests and the prevalences of each outcome in the present study are presented in Table II.

TABLE II. Classification of diabetics with CPAF and alcohol tests, and the prevalence (pro cents) of each group. (+) = Positive to the CPAF criterion. (0) = Negative to the CPAF criterion. Criterion I = A visible facial flush. Criterion II = An increase in skin temperature $\geq 1.0^{\circ}\text{C}$. Criterion III = An increase in plasma acetaldehyde concentration $\geq 4 \mu\text{mol/l}$

	CPAF test			Alcohol test			Prevalence (pro cents)
	Criterion I	Criterion II	Criterion III	Criterion I	Criterion II	Criterion III	
CPAF positive	+	+	+ / 0	0	0	0	35
	+	+ / 0	+	0	0	0	
CPAF positive and alcohol flusher	+	+ / 0	+	+	+	0	13
Falsely CPAF positive alcohol flusher	+	+	0	+	+	0	5
CPAF negative	0	+ / 0	0	0	0	0	47
	0	0	+ / 0	0	0	0	

The present results do not support the suggestion that CPAF is more commonly seen in diabetics with a known family history of the disease [14], but it confirmed a difference in CPAF reaction pattern between the sexes [18]. As we earlier reported [7, 8] CPAF was more common in patients continuously treated with chlorpropamide than after the single-dose exposure to the drug. The CP concentrations were determined in some of these subjects and the results were in accordance with our earlier findings.

In conclusion we have shown that, using registration of facial skin temperature and determination of plasma acetaldehyde in combination with the visible response in the CPAF test, it is possible to evaluate the test objectively. However, in order to detect subjects with falsely positive CPAF tests, alcohol tests without chlorpropamide premedication must be carried out in the flushers not having elevated acetaldehyde levels.

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Facial Skin Temperature in Chlorpropamide Alcohol Flush

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ABSTRACT. Jerntorp P. (Department of Medicine, University of Lund, Malmö General Hospital, Malmö, Sweden.) Facial skin temperature in chlorpropamide alcohol flush. *Acta Med Scand* . . .

Objective methods to study the chlorpropamide alcohol flush (CPAF) have been inadequate. Determination of blood acetaldehyde has proved to be a promising method, but the analysis is difficult and time-consuming. To measure the facial skin temperature is more handy. The results of these measurements can be presented as δT (skin temperature increase), %T (per cent of maximum possible temperature rise) or $\delta MT CI$ (malar thermal circulation index) after calculations. The baseline skin temperature is accounted for in %T and $\delta MT CI$. Blood acetaldehyde determinations and placebo-alcohol tests can be used to separate the CPAF reaction from alcohol flushing. Single dose CPAF tests including facial skin temperature measurements were performed in 133 type 2 (non-insulin dependent) diabetics. Facial flush was observed in 42.9%. The specificity and sensitivity of all three skin temperature methods were high: 88.2, 85.5, 96.1%, and 89.5, 86.0, 86.0%, respectively. Skin temperature measurement, whether expressed as δT , %T or $\delta MT CI$, provides a method to study CPAF with high accuracy. *Key words:* alcohol, chlorpropamide, chlorpropamide alcohol flush, malar thermal circulation index, skin temperature, type 2 (non-insulin dependent) diabetes mellitus.

The chlorpropamide alcohol flush (CPAF), a side-effect of chlorpropamide medication, has been claimed to be genetically determined in type 2 (non-insulin dependent) diabetes (1). A low prevalence of late diabetic complications has been found in those with a positive CPAF reaction compared to non-flushers (2–5). However, the prevalence of CPAF differed considerably between studies (6), and the difference in prevalence of vascular diseases between flushers and non-flushers could not be confirmed in all studies (7). Clearly, objective methods are necessary to investigate the predictive value of the CPAF reaction. Determinations of blood acetaldehyde levels have been successfully used (8–10). Measurement of the increase in facial skin temperature (δT) during the CPAF test, being more handy, was used in some studies (10). However, the malar thermal circulation index ($\delta MT CI$) was claimed to be a more physiological method to interpret the temperature changes (11). The method of expressing the skin temperature changes as per cent of maximum possible temperature rise (%T) was recently introduced (12).

In this study these three skin temperature methods are compared regarding their specificity and sensitivity in CPAF tests. The importance of placebo-alcohol tests, and how to interpret the combined result of these two challenge tests using plasma acetaldehyde determinations, are also discussed.

STUDY POPULATION AND METHODS

We studied 133 type 2 (non-insulin dependent) diabetics, 64 females and 69 males, attending our Out-patient Clinic. Their median age was 68 years (range 16–81) and the mean duration after the diagnosis of diabetes was 9 years (range 1–20). All patients were on a regulated diet; 49 patients received additional treatment with glibenclamide or glipizide and 17 with insulin after several years of sulfonylurea treatment.

Abbreviations: CPAF = chlorpropamide alcohol flush, δT = skin temperature increase, %T = per cent of maximum possible temperature rise, $\delta MT CI$ = malar thermal circulation index.

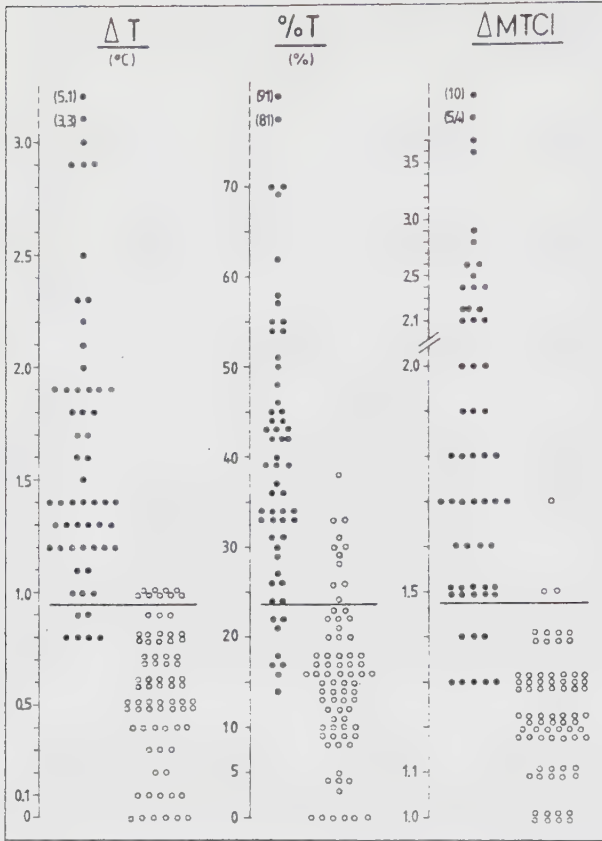


Fig. 1. Results from 133 CPAF tests expressed as δT (skin temperature increase), %T (per cent of maximum possible temperature rise) and $\delta MTCI$ (malar thermal circulation index). ●=CPAF-positive ($n=57$), ○=CPAF-negative ($n=76$). The horizontal line represents the lower limit for a positive test defined for each method.

All patients underwent a standardized CPAF test, 73 being pretreated with a single dose of 250 mg and 60 with 375 mg chlorpropamide (Diabinese[®], Pfizer Corp.) 12 hours before ingestion of 8 g alcohol. All other medication was withdrawn 24 hours before the test. The tests were performed at a constant room temperature of 22°C and the facial skin temperature was measured every 5 min 2 cm below the outer canthus of the left eye with a sensitive probe (Electrolab, Cph). The baseline skin temperature had to be stable before alcohol administration and the measurements were continued for another 30 min. The appearance or absence of facial flush was observed by one and the same person during all tests. The results were transferred into δT , %T and $\delta MTCI$.

$\delta T = T_{30} - T_b$, where T_{30} is the skin temperature after 30 min and T_b is the baseline temperature (10),

$\%T = (T_{30} - T_b) / (36.5 - T_b) \times 100$, where 36.5 is the maximal normal possible facial skin temperature (12),

$\delta MTCI = (T_{30} - 22) \times (37 - T_b) / (37 - T_{30}) \times (T_b - 22)$, where 22 is the ambient and 37 the core temperature (11).

Table I. Specificity and sensitivity (%) of a positive CPAF test of three skin temperature methods

	δT	%T	$\delta MTCI$
Specificity	88.2	85.5	96.1
Sensitivity	89.5	86.0	86.0

The criterion for a positive CPAF reaction was selected for each of the three methods: $\delta T = 1.0^\circ\text{C}$ or more (10), $\%T = 24\%$ or more (from present results), $\delta\text{MTCI} = 1.5$ or more (11).

Student's *t*-test was used for statistics. All data are presented as mean $\pm$ SEM.

RESULTS

In 57 (42.9%) of the 133 CPAF tests the patients were judged as flushers. No significant differences were found between patients tested after 250 or 375 mg chlorpropamide or between females and males. The following results were obtained from the three skin temperature methods for flushers and non-flushers. δT : 1.7 ± 0.1 and $0.6 \pm 0^\circ\text{C}$, $\%T$: 40.0 ± 2.2 and $14.9 \pm 1.0\%$, δMTCI : 2.1 ± 0.2 and 1.2 ± 0 , respectively ($p < 0.001$).

The individual results, expressed as δT , $\%T$ and δMTCI , are presented in Fig. 1. The specificity and sensitivity of each of the three methods are presented in Table I.

DISCUSSION AND CONCLUSION

The δT , $\%T$ and δMTCI methods were all highly specific and sensitive when used to study the CPAF reaction. Although the δT method does not account for baseline skin temperature, it showed as high specificity and sensitivity as the $\%T$ and δMTCI methods in this study. δT might still be the best one, not only because of very simple calculation, but also because only 31 of 133 tests were overlapping, while there were 73 and 61 overlaps, respectively, when $\%T$ and δMTCI were used. Furthermore, in most studies the lower limit for a positive test with the δT method was set to 1.0°C . The result of this study shows a specificity of 100% and a sensitivity of 84.2% if, instead, the criterion for a positive test was set to 1.1°C or more (Fig. 1). Such effects could not be gained by the other two methods, and the sensitivity of any of the methods could not become higher without losing substantially in specificity.

Using a skin temperature method together with plasma acetaldehyde determinations and placebo-alcohol tests, CPAF could be studied with high accuracy (10). Having a sensitivity of 86% and a specificity of 85%, plasma acetaldehyde determination does not seem to be superior to the skin temperature methods (13). However, the importance of acetaldehyde determinations is that an increased level of this intermediate metabolite of ethanol indicates a chlorpropamide-induced inhibition of aldehyde dehydrogenase (14). Thus, irrespectively of the reaction during the placebo-alcohol test, increased plasma acetaldehyde levels and flushing during a chlorpropamide-alcohol challenge strongly indicate a positive CPAF test. On the other hand, flushing without increase in plasma acetaldehyde during both CPAF and placebo-alcohol tests is typical of the common alcohol flush (10).

In conclusion, facial skin temperature measurement, whether expressed as δT , $\%T$ or δMTCI , provides a specific and sensitive method in studying facial flushing and the prevalence of CPAF. Placebo-alcohol tests and blood acetaldehyde determinations should be used to separate CPAF from alcohol flushing.

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Plasma Chlorpropamide: A Critical Factor in Chlorpropamide-Alcohol Flush

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Summary. The chlorpropamide-alcohol flush (CPAF) phenomenon was quantitatively related to blood levels of acetaldehyde and chlorpropamide in 105 Type II diabetics, of whom 74 had not previously taken the drug and 31 were on chronic treatment. Standardized skin temperature recordings were made with a sensitive probe. Plasma ethanol and acetaldehyde concentrations were determined by gas chromatography, and those of chlorpropamide by high-pressure liquid chromatography. There were significant positive correlations between plasma acetaldehyde and the skin temperature increase, between plasma chlorpropamide and plasma acetaldehyde, and between plasma chlorpropamide and the skin temperature increase. CPAF-positive patients became CPAF-negative and vice versa following reduction and increase, respectively, in the dose of chlorpropamide. Thus, the CPAF reaction is a consequence of chlorpropamide inhibition of the oxidation of ethanol-generated acetaldehyde, and it appears that the plasma concentration of chlorpropamide is critical. It remains an open question whether the CPAF test has any prognostic value.

Key words: chlorpropamide, alcohol; alcohol-induced flush, plasma chlorpropamide, plasma alcohol, plasma acetaldehyde

less prone to develop vascular complications than non-flushers [2–5]. However, the CPAF frequency has shown great variation in different studies [6, 7], and preliminary results indicate that the blood level of chlorpropamide may be a critical factor in CPAF [8].

It has long been suspected that CPAF is mediated by chlorpropamide blockade of the oxidation of acetaldehyde, the intermediate metabolite of ethanol [9], and it has recently been demonstrated that blood levels of acetaldehyde are higher in CPAF-positive than in CPAF-negative subjects [10]. The present study quantitated the CPAF phenomenon in relation to blood levels of acetaldehyde and chlorpropamide. The findings support the view that CPAF is a consequence of chlorpropamide-induced inhibition of acetaldehyde turn-over, and that the blood concentration of chlorpropamide may be a critical factor in the CPAF reaction.

Subjects and Methods

Patients

The study comprised 105 patients with Type II diabetes, 53 females and 52 males, aged 16–83 years, weight 90–150% of the ideal. The mean duration of the disease after diagnosis was 9 years (range 1–22 years). All patients regularly visited the Diabetes Care Unit, Malmö General Hospital. All patients had a regulated diet. In addition, 5 were on regular insulin treatment and 31 were on chlorpropamide, 125–375 mg once daily. In those cases, the CPAF test was carried out without alteration of their chlorpropamide or insulin treatment. In 28 patients who were on treatment with glipizide or glibenclamide, the drug was withdrawn at least 24 h before the

Many, but not all chlorpropamide-treated Type II diabetics display and experience facial flushing after intake of a small amount of ethanol, the so-called chlorpropamide-alcohol flushing (CPAF) reaction [1]. It has been suggested that the CPAF reaction pattern (positive or negative) is genetically determined and that it has prognostic significance, flushers being

Table 1. Occurrence of positive CPAF reactions in patients given a single dose of chlorpropamide and in patients on chronic chlorpropamide-treatment; and increase in facial skin temperature (mean $\pm$ SEM) during CPAF test, 30 min after the intake of ethanol (ΔT_{30})

	Number of patients tested	<i>n</i>	CPAF-positive ΔT_{30} [$^{\circ}$ C]	<i>n</i>	CPAF-negative ΔT_{30} [$^{\circ}$ C]	<i>p</i>
Single dose	74	31 (42%)	2.0 $\pm$ 0.2	43 (58%)	0.6 $\pm$ 0.3	< 0.001
Chronic therapy	31	28 (90%)	2.4 $\pm$ 0.2	3 (10%)	0.6 $\pm$ 0.1	< 0.005
Total	105	59 (56%)	2.2 $\pm$ 0.2	46 (44%)	0.6 $\pm$ 0.1	< 0.001

Table 2. Plasma ethanol concentration (mean $\pm$ SEM) and increase in plasma acetaldehyde concentration (mean $\pm$ SEM) during CPAF tests

	Number of patients tested	Plasma ethanol [mmol/l]		<i>p</i>	Δ Plasma acetaldehyde [μ mol/l]		<i>p</i>
		CPAF-positive	CPAF-negative		CPAF-positive	CPAF-negative	
Single dose	49	4.0 $\pm$ 0.3	2.5 $\pm$ 0.4	< 0.005	6.0 $\pm$ 0.9	2.5 $\pm$ 0.3	< 0.001
Chronic therapy	29	3.4 $\pm$ 0.4	2.7 $\pm$ 0.9	N.S.	13.3 $\pm$ 2.4	3.3 $\pm$ 0.3	N.S.
Total	78	3.7 $\pm$ 0.3	2.5 $\pm$ 0.3	< 0.005	9.7 $\pm$ 1.4	2.6 $\pm$ 0.2	< 0.001

CPAF test. Any possible influence of remaining glipizide or glibenclamide was excluded, as preceding alcohol challenge in these patients showed no elevation of plasma acetaldehyde (see below).

CPAF Test

The chlorpropamide-alcohol flush (CPAF) test was carried out as described by Lesley and Pyke [1], although pure ethanol (8 g) in fruit juice was used instead of sherry. The ethanol was always administered in the morning after an adjustment period of 20–30 min, and the temperature measurements and flush judgements were carried out before and after 30 min. In patients not on chlorpropamide treatment ($n = 74$), a dose of chlorpropamide 250 or 375 mg (Diabines(e)[®], Pfizer Corp., Groton, Conn., USA) was given on the preceding evening, 12 h before the administration of ethanol. In subjects already on chlorpropamide, the normal dose was usually taken in the morning, shortly before ethanol administration.

Skin temperature was recorded every 5 min with a sensitive probe (Electrolab., Copenhagen), 2 cm below the lateral canthus of the left eye.

A positive flush reaction was considered to be present when the examiner noted facial redness of the subject tested, and/or when the increase in facial skin temperature was more than 1.0 $^{\circ}$ C.

Blood Sampling

Blood samples for measurement of the concentration of ethanol, acetaldehyde and chlorpropamide

were obtained by venepuncture immediately before and 25 min after ethanol administration.

Chemical Analyses

Ethanol and acetaldehyde concentrations were determined by gas chromatography [12], and those of chlorpropamide by high-pressure liquid chromatography [11].

Calculations

Regression analysis was carried out by the method of least squares. The statistical significance of differences was assessed by Student's *t*-test or by chi square analysis.

Results

Occurrence of CPAF

As shown in Table 1, 56% of subjects had a positive CPAF test. Of the 31 patients on maintenance therapy, 90% were positive, whereas significantly fewer (42%; $p < 0.001$) of the 74 patients not previously exposed to chlorpropamide were positive. Of the females, 62% were positive, whilst 50% of the males were positive (ns).

Changes in Skin Temperature

As further shown in Table 1, the increase in skin temperature 30 min after alcohol ingestion (ΔT_{30}) was

Table 3. Plasma chlorpropamide concentrations (mean ± SEM) during CPAF tests

	Number of patients tested	Plasma chlorpropamide [µg/ml]				p
		CPAF-positive	n	CPAF-negative	n	
Single dose	74	34.6 ± 1.7	31	27.7 ± 1.3	43	< 0.005
Chronic therapy	31	92.2 ± 5.4	28	80.0 ± 17.1	3	NS

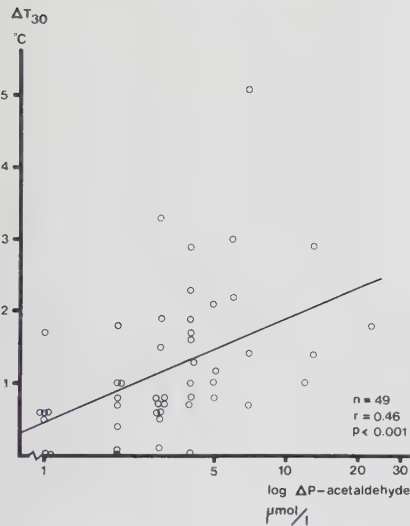


Fig. 1. Skin temperature increase at 30 min (ΔT_{30}) versus log of difference in plasma acetaldehyde in patients given a single dose of chlorpropamide

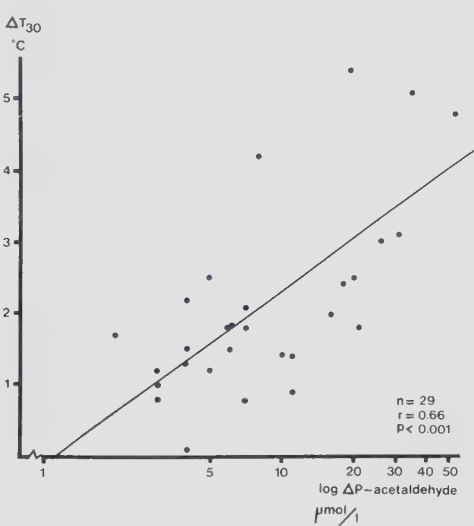


Fig. 2. Skin temperature increase at 30 min (ΔT_{30}) versus log of difference in plasma acetaldehyde in patients chronically treated with chlorpropamide

greater in CPAF-positive than in CPAF-negative patients.

Acetaldehyde and Ethanol Concentrations

The concentrations of acetaldehyde and ethanol were higher in CPAF-positive than in CPAF-negative patients (Table 2).

Chlorpropamide Concentrations

The patients on chronic chlorpropamide therapy had much higher drug concentrations than those not previously exposed to it (Table 3; $p < 0.001$), and among subjects given a single chlorpropamide dose, those with a positive CPAF test had a significantly higher chlorpropamide concentration ($p < 0.005$) than those with a negative test. On the other hand, CPAF-posi-

tive subjects given a single dose had significantly lower chlorpropamide concentrations than the CPAF-negative patients on chronic chlorpropamide treatment.

Correlations Between Change in Skin Temperature and Plasma Concentration of Acetaldehyde and Chlorpropamide

As shown in Figs. 1–6, there was a significant correlation between the increase in skin temperature and the acetaldehyde concentration, between the latter and the plasma concentration of chlorpropamide, and between the latter and the change in skin temperature. This was true irrespective of whether comparisons were made on the entire series, or after subdivision according to the type of chlorpropamide exposure.

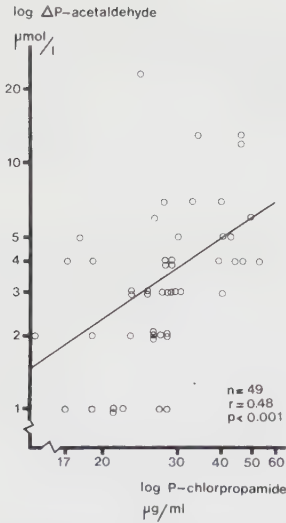


Fig. 3. Log of difference in plasma acetaldehyde versus log plasma concentration of chlorpropamide in subjects exposed to a single dose of chlorpropamide

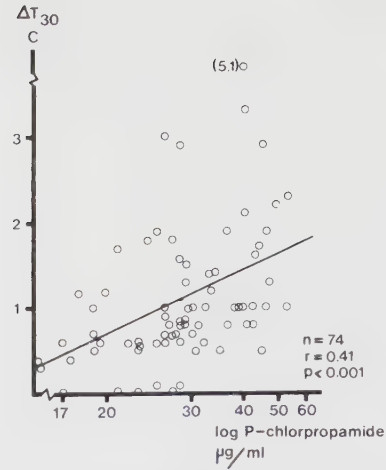


Fig. 5. Difference in skin temperature at 30 min (ΔT_{30}) versus log serum concentration of chlorpropamide in patients given a single dose of chlorpropamide

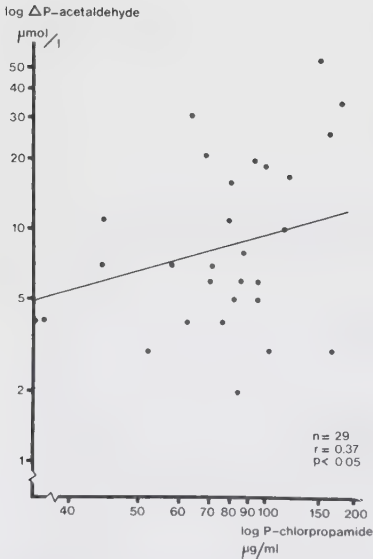


Fig. 4. Log of difference in plasma acetaldehyde versus log serum concentration of chlorpropamide in patients chronically treated with chlorpropamide

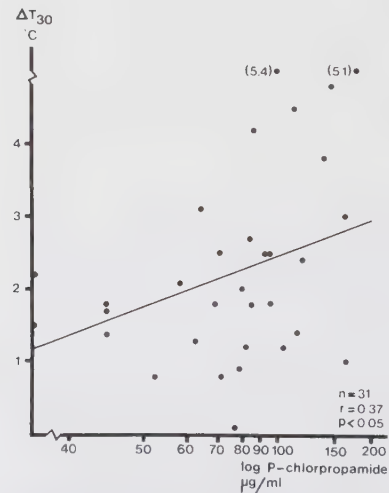


Fig. 6. Difference in skin temperature at 30 min (ΔT_{30}) versus log serum concentration of chlorpropamide in patients chronically treated with chlorpropamide

Table 4. Alterations in CPAF reaction pattern following change in chlorpropamide treatment

Patient	Chlorpropamide dose	Plasma chlorpropamide [µg/ml]	Change in plasma acetaldehyde [µmol/l]	Δ T ₃₀ [°C]	CPAF + / -
A: female	single dose:				
	0	0	2	0.7	—
	62.5 mg	13	4	0.7	—
	125 mg	17	3	1.1	+
	250 mg	28	4	1.6	+
	375 mg	59	7	3.5	+
B: male	days after withdrawal of 375 mg daily:				
	1	164	26	3.0	+
	2	152	24	2.4	+
	3	114	14	2.3	+
	10	18	4	0.7	—

Alteration in CPAF Reaction Pattern Following Change in Chlorpropamide Treatment

Two examples of altered CPAF reaction pattern following changes in chlorpropamide administration are shown in Table 4. The female patient A was being treated only by dietary regulation, and she underwent CPAF tests after administration of various single doses of chlorpropamide. It can be seen that although there was no CPAF reaction when only 62.5 mg was given, a positive CPAF reaction occurred following doses of 125, 250 and 375 mg. It is apparent, too, that there was a concomitant and parallel increase in the plasma chlorpropamide and plasma acetaldehyde levels and in skin temperature.

In the male patient B, who had been on chlorpropamide 375 mg daily, CPAF tests were carried out after drug withdrawal. In him the CPAF reaction was positive for a period of 3 days while the plasma chlorpropamide concentration remained above 100 µg/ml, but it became negative after 10 days, when the chlorpropamide concentration had fallen to 18 µg/ml. There were parallel reductions in plasma acetaldehyde concentration and in the increase in skin temperature.

Discussion

In agreement with a previous study [10], acetaldehyde concentrations were higher among CPAF-positive than CPAF-negative patients, and the acetaldehyde concentration was well correlated with the increase in skin temperature. This supports the concept that the CPAF reaction is a consequence of acetaldehyde accumulation, secondary to chlorpropamide-induced inhibition of the oxidation of ethanol-generated acetaldehyde. Hence it seems logical to sus-

pect that chlorpropamide concentration is a critical factor in the CPAF reaction, and the following findings lend credence to this view.

First, the CPAF-positive frequency was higher among patients chronically treated with chlorpropamide, and who therefore had a higher plasma chlorpropamide concentration, than among those not previously exposed to the drug, and who therefore had a much lower level. Second, there was a correlation between the plasma concentrations of chlorpropamide and acetaldehyde and the increase in skin temperature.

It must be noted, on the other hand, that chlorpropamide concentrations in those patients who were CPAF-positive after a single dose of chlorpropamide were lower than in the few CPAF-negative patients who had been chronically treated with chlorpropamide. However, this need not contradict the concept that the chlorpropamide concentration is a major determinant in the CPAF reaction; rather, it indicates that the critical chlorpropamide level differs between individuals. That the chlorpropamide level is indeed a critical factor is apparent from the examples showing that a CPAF-negative patient can become CPAF-positive, and vice versa, simply by a change in the degree of exposure to chlorpropamide.

In summary, the CPAF reaction is a consequence of chlorpropamide-induced inhibition of the oxidation of acetaldehyde generated from ethanol. The plasma concentration of chlorpropamide is critical; a CPAF-negative subject can become CPAF-positive, and vice versa, simply by a change in the dose of chlorpropamide. The critical level differs between individuals. It remains an open question, therefore, whether the CPAF test has any prognostic value.

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ON PLASMA CHLORPROPAMIDE, BODY WEIGHT AND SEX DIFFERENCE IN CHLORPROPAMIDE ALCOHOL FLUSH (CPAF)

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Summary - There is evidence that the plasma chlorpropamide (CP) concentration is an important determinant of the chlorpropamide alcohol flush (CPAF). In 74 type 2 diabetics a highly significant correlation ($r = 0.51$, $p < 0.001$) was present between the levels of plasma CP and the appearance of CPAF, measured as an index (the sum of the increase of the skin temperature and one-fourth of plasma acetaldehyde increase). Lower or no correlations were found when different threshold levels were considered. Body weight was significantly ($p < 0.001$) inversely correlated to plasma chlorpropamide levels, which resulted in higher CP levels in females (mean body weight 68.6 kg, CP concentration 34.4 mg/l) than in males (83.4 kg, 26.0 mg/l), which explains the female preponderance among flushers.

INTRODUCTION

The chlorpropamide alcohol flush (CPAF), a phenomenon common in chlorpropamide treated type 2 (non-insulin dependent) diabetics, was suggested to be associated with few late vascular complications in diabetes mellitus (1-4). Subsequently, this view has been challenged, and the frequency of CPAF has been found to vary greatly between different studies (5-7). In addition, a sex difference in CPAF frequency has been reported (8, 9). A possible explanation of these discrepancies is that the frequency of CPAF depends upon the plasma level of chlorpropamide (10). Indeed, a possible correlation has been found between plasma chlorpropamide (CP) and the increase in skin temperature (11-13). In addition, non-flushers can become flushers, and vice versa following increase and reduction in CP dosage (11, 12). Recently, it was suggested that the relation between plasma CP and the appearance of CPAF is present only at drug levels below 40 mg/l, indicating a "threshold effect" (14).

Thus, plasma CP is a critical factor determining CPAF. To further elucidate this relationship body weight and sex were compared to plasma CP levels and the CPAF reaction, respectively, and the correlation between plasma CP and the appearance of CPAF was determined at different CP levels.

PATIENTS AND METHODS

The study comprised 74 type 2 diabetics, 41 females and 33 males, aged 16-80 years, and with 90-150% of the ideal weight, and a mean duration after diagnosis of 8 years (range 1-18 years).

Test procedure

A standard dose of CP 250 mg was given in the evening 12 h before the test. Pure ethanol (8 g) in fruit juice was administered in the next morning after an adjustment of 30 min. Skin temperature was recorded every 5 min with a sensitive probe, 2 cm below the lateral canthus of the left eye, before and 30 min after the alcohol intake. Flush judgements were done by the same examiner in all probands. Blood samples for measurements of plasma CP (n=74) and acetaldehyde (n=49) concentrations were obtained by venepuncture before and 25-30 min after the alcohol ingestion, the time of maximal flushing in most subjects (15).

Plasma chlorpropamide

Chlorpropamide concentrations were determined by high-pressure liquid chromatography (16).

Plasma acetaldehyde

After hydrolysis with perchloric acid, acetaldehyde concentrations were determined in plasma by gas chromatography of head space samples (15).

CPAF Index

The CPAF test was considered to be positive if a visible flush was observed by the examiner. However, to assess the correlation between plasma CP levels and the appearance of CPAF more objectively a CPAF Index was designed. In previous reports a skin temperature increase

of $\geq 1.0^{\circ}\text{C}$ (usually less than 5°C) and plasma acetaldehyde increase of $\geq 4 \mu\text{mol/l}$ (usually less than $20 \mu\text{mol/l}$) during CPAF test independently were considered to be indicators of the flush reaction (11). Consequently for each proband CPAF Index was calculated as the sum of the temperature increase and one-fourth of the acetaldehyde increase.

Statistics

Chi-square test and Student's t-test were used to assess statistical significance. Regression analyses were carried out by the method of least squares. Data are given as mean $\pm$ SEM.

RESULTS

A flush reaction (positive CPAF test) was seen in 42% (31/74) of the probands, more commonly in females (21/41) than in males (10/33), but this difference was not statistically significant.

Body weight

Females had significantly lower body weight than males (Table I). CPAF positive females and males had slightly lower body weight than CPAF negative females and males, respectively (n.s.) The flushers had a mean body weight of 71.5 ± 2.5 kg and the non-flushers 77.9 ± 2.5 kg (n.s.)

Plasma chlorpropamide concentrations

Females had significantly higher levels of CP than males (Table II). CPAF positive females and males had higher plasma levels of CP compared to CPAF negative females and males, respectively ($p < 0.05$). CP levels were significantly higher in flushers than in non-flushers: 34.6 ± 1.7 mg/l and 27.7 ± 1.3 mg/l ($p < 0.005$).

Correlation between body weight and plasma chlorpropamide concentration.

There was a significant negative correlation between body weight and plasma levels of CP ($p < 0.001$). (Fig. 1.)

Table I. Body weight (kg) in females compared to males in relation to the CPAF reaction (mean $\pm$ SEM).

	Body weight (kg)				p
	Females	(n)	Males	(n)	
CPAF positive	66.4 $\pm$ 1.9	(21)	82.2 $\pm$ 5.4	(10)	<0.005
CPAF negative	71.0 $\pm$ 3.4	(20)	83.9 $\pm$ 3.1	(23)	<0.01
Total	68.6 $\pm$ 1.9	(41)	83.4 $\pm$ 2.7	(33)	<0.001

Table II. Plasma levels of chlorpropamide (mg/l) in females compared to males in relation to CPAF (mean $\pm$ SEM).

	Plasma chlorpropamide concentration (mg/l)				p
	Females	(n)	Males	(n)	
CPAF positive	37.3 $\pm$ 1.9	(21)	29.0 $\pm$ 2.9	(10)	<0.05
CPAF negative	31.3 $\pm$ 2.1	(20)	24.7 $\pm$ 1.2	(23)	<0.01
Total	34.4 $\pm$ 1.5	(41)	26.0 $\pm$ 1.3	(33)	<0.001

Table III. Correlations between CPAF Index (the sum of increase in skin temperature and one-fourth of plasma acetaldehyde increase) and plasma chlorpropamide level (mg/l) at 5 different drug levels in 49 (25 flushers and 24 non-flushers) type 2 diabetics.

Plasma chlorpropamide level (mg/l)	Correlation between CPAF Index and plasma chlorpropamide concentration (mg/l)		
	n	r	p
<53	49	0.51	<0.001
<40	40	0.41	<0.01
>40	9	0.10	n.s.
<33	36	0.19	n.s.
>33	13	0.06	n.s.

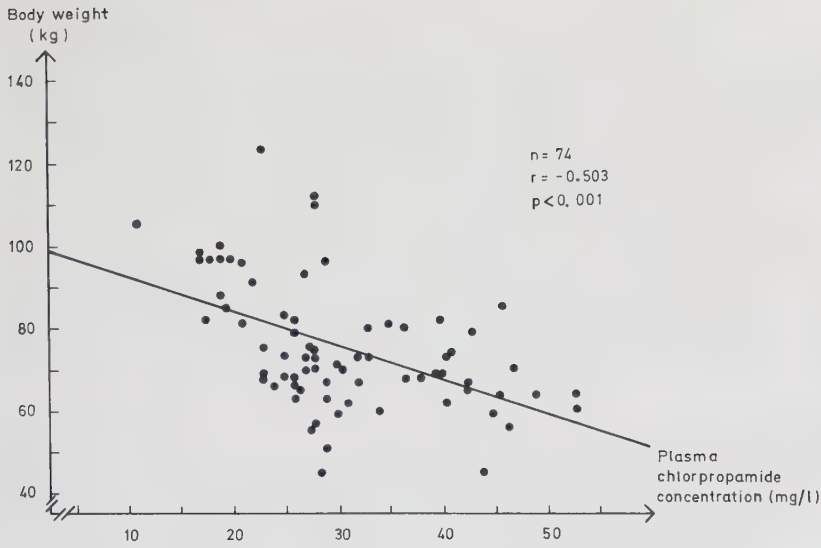


Fig. 1. Correlation between body weight (kg) and plasma levels of chlorpropamide (mg/l) in 74 type 2 diabetics.

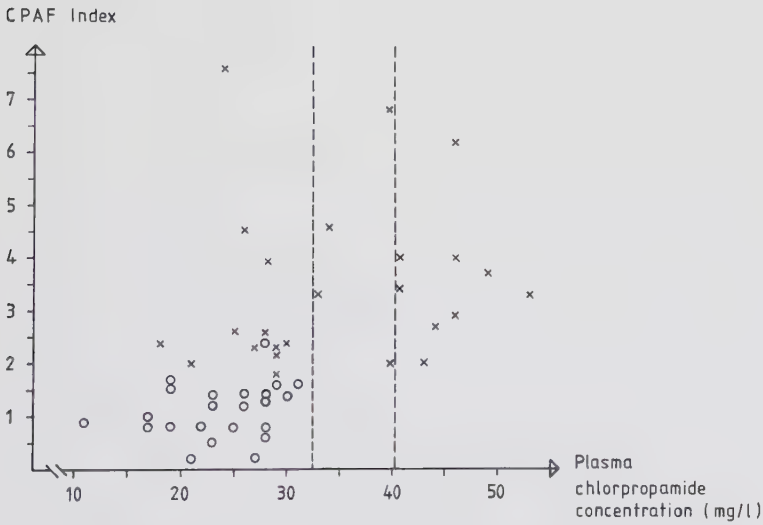


Fig. 2. CPAF Index (the sum of skin temperature increase and one-fourth of plasma acetaldehyde increase) in relation to plasma chlorpropamide concentrations (mg/l) in 25 flushing (x) and 24 non-flushing (o) type 2 diabetics. The vertical lines of short dashes represent the differentiating chlorpropamide levels used for determination of possible correlations between CPAF Index and levels of chlorpropamide.

Correlations between appearance of CPAF and plasma chlorpropamide concentrations at different drug levels.

In order to find a possible "threshold effect" correlations were calculated between the appearance of CPAF (CPAF Index) and plasma levels of CP at 5 different drug levels (Fig.2 and Table III). The correlation was highly significant when no limitations of the CP levels were made ($p < 0.001$). With the exception of one proband without a visible facial flush the CPAF Index completely separated flushers from non-flushers (Fig. 2).

DISCUSSION

The results of this study confirm that at all plasma CP levels there is a correlation between the appearance of CPAF and the level of CP. A CPAF Index was used to objectively present the flush. This was possible since we had shown correlations between skin temperature and CP concentration, and between the latter and plasma levels of acetaldehyde, the intermediate metabolite of ethanol, in the same probands (11). This study was not able to confirm the suggestion of a "threshold effect" of plasma CP in CPAF (14). The positive correlation between CPAF and levels of CP was reduced when an upper limit was introduced at 40 mg/l. Indeed, when an upper limit was proposed at 33 mg/l there were no correlation at all (Table III). Moreover, the presence of a "threshold effect" would have been unique for a drug-induced enzyme inhibition.

It has recently been shown that the levels of plasma CP is not the sole determinant of CPAF. The increase in blood acetaldehyde concentrations, a result of aldehyde dehydrogenase inhibition, is probably also determined by the basal aldehyde dehydrogenase activity (17).

The results confirmed the finding of a negative correlation between body weight and plasma levels of CP (18). Since the CP level is an important determinant in CPAF it would seem important to adjust the CP dosage to the body weight of the proband. This has also been suggested for the amount of alcohol given when testing for CPAF (13). The sex difference reported in CPAF among type 2 diabetics (8, 9) could be explained by the body weight dependence of plasma CP.

In conclusion, plasma chlorpropamide is a critical factor, but not the sole determinant, in CPAF. The suggested "threshold effect" of plasma CP in CPAF was not confirmed. The negative correlation between body weight and plasma CP levels suggests an explanation for the reported higher frequency of female than male flushers.

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Chlorpropamide-alcohol flushing, aldehyde dehydrogenase activity, and diabetic complications

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Abstract

Many diabetics who take chlorpropamide (a sulphonyl-urea compound) experience facial flushing after drinking even small amounts of alcohol. These flushers have a noticeably lower prevalence of late complications of diabetes (microangiopathy, macroangiopathy, and neuropathy) than non-flushers. This flush reaction is accompanied by increased blood acetaldehyde concentrations, suggesting an inhibition of aldehyde dehydrogenase activity. In the present study the activity of this enzyme in erythrocytes was assessed in the absence of chlorpropamide. Erythrocyte homogenates obtained from flushers and non-flushers were incubated with acetaldehyde and the rate of metabolism studied. Flushers eliminated acetaldehyde more slowly at a low range of concentrations (0-30 $\mu\text{mol/l}$), suggesting a difference in aldehyde dehydrogenase activity. Further studies are needed to clarify the role of this enzyme in the pathogenesis of diabetic complications.

Introduction

For 25 years facial flushing after intake of small amounts of

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alcohol has been a well-known side effect of chlorpropamide medication,¹ a sulphonylurea derivative used in the treatment of type II diabetes (non-insulin-dependent or maturity-onset diabetes). The implications of the flush were not known until 1978, however, when Leslie and Pyke² reported that chlorpropamide-alcohol flushing was inherited as an autosomally dominant trait in type II diabetes. This was followed by reports of a lower prevalence of diabetic retinopathy,³ large-vessel disease,^{4, 6} peripheral neuropathy,⁵ and diabetic nephropathy⁶ in the flushers than in the non-flushers. The prevalence of chlorpropamide-alcohol flushing in type II diabetes has varied considerably at around 30%.

Since the flush reaction seemed to be related to the pathogenesis of angiopathy, studies of its biochemical basis were warranted. We found that flushers had higher blood concentrations of acetaldehyde, the first metabolite of ethanol, than non-flushers during a chlorpropamide-alcohol challenge test.⁷ We also found higher concentrations of chlorpropamide in flushers than in non-flushers,⁸ which accords with the fact that chlorpropamide-induced inhibition of aldehyde dehydrogenase leads to a rise in blood acetaldehyde concentrations.⁹ Barnett *et al*¹⁰ did not detect this difference in chlorpropamide concentrations between flushers and non-flushers, although they confirmed our finding that acetaldehyde concentrations were increased during the flush. This discrepancy can be explained by the different size of the populations studied (105 subjects in our study⁸ and 21 in theirs¹⁰). Furthermore, we found a considerable overlap in serum chlorpropamide concentrations, whereas the difference in blood acetaldehyde concentrations was highly significant with a minimal overlap. Obviously other factors might be of importance. This prompted us to study the role of aldehyde dehydrogenase in diabetes.

Acetaldehyde is converted to acetate by aldehyde dehydrogenase, which is distributed in various organs, the highest activity being found in the liver.¹¹ Two major isoenzymes have been isolated from mammalian livers; one is mitochondrial with a low K_m for aldehydes, the other is cytosolic with a high K_m for aldehydes.¹² Inoue *et al*¹³ isolated aldehyde dehydrogenase from human erythrocytes and found that it shared several characteristics with the cytosolic enzyme. It had a high K_m for aldehydes, was strongly inhibited by disulfiram, and had a biphasic nature of kinetics which suggested both high and low affinity sites for acetaldehyde.¹⁴

Patients and methods

Ten type II diabetics with chlorpropamide-alcohol flushing (eight

women and two men) and 10 type II diabetics without (five women and five men) were studied. The mean age for the two groups was 69 and 62 years respectively, the mean duration after the diagnosis of diabetes six and nine years respectively, and the mean body weight 117% and 123% of ideal weight respectively. None of the differences was statistically significant. Five flushers and four non-flushers were treated with diet only whereas five flushers and six non-flushers were treated with glibenclamide or glipizide. All diabetic treatment was stopped for at least 24 hours before any part of the study and the patients were told to refrain from alcohol before the test.

CHALLENGE TEST

The patients were studied with a standardised chlorpropamide-alcohol flushing challenge test. Twelve hours after taking 250 mg chlorpropamide the patients were given 8 g of alcohol in fruit juice. The flush reaction was assessed by measuring skin temperature and determining blood acetaldehyde concentrations.^{2,7} One month later when no chlorpropamide metabolites remained from the challenge test blood samples were collected to study aldehyde dehydrogenase activity.

ALDEHYDE DEHYDROGENASE ACTIVITY

To assess the activity of erythrocyte aldehyde dehydrogenase in flushers and non-flushers we used the method of Inoue *et al*¹⁵ slightly modified as follows. Blood samples were collected from the patients in the morning in heparinised vacuum tubes, kept at 4°C, and within one hour centrifuged at $1000 \times g$ at 4°C for 10 minutes. Plasma and erythrocytes were mixed in fixed proportions (5 : 1) and homogenised by freezing and thawing. Three millilitres of the homogenate was incubated at 37°C with 100 μmol acetaldehyde/l and 1 mmol nicotinamide-adenine dinucleotide (NAD)/l in 5-ml airtight glass vials. Samples were taken at fixed intervals and the reaction stopped by cooling the vials in iced water for 10 minutes followed by deproteinisation with 0.5 ml 35% perchloric acid. After centrifugation at 4°C the clear supernatant was assayed for acetaldehyde using head-space gas chromatography.⁷

Student's *t* test was used to analyse data from the challenge test and Wilcoxon's test for the data on aldehyde dehydrogenase activity.

Results

During the chlorpropamide-alcohol flushing challenge test the flushers had positive reactions and the non-flushers did not. The mean increase in facial skin temperature was $2.2 \pm 0.3^\circ\text{C}$ and $0.5 \pm 0.1^\circ\text{C}$ for the flushers and non-flushers respectively ($p < 0.001$). The mean increase in blood acetaldehyde concentration 25 minutes after the intake of 8 g of ethanol was $6.3 \pm 1.6 \mu\text{mol/l}$ and $1.6 \pm 0.2 \mu\text{mol/l}$

respectively ($p < 0.01$) for the flushers and non-flushers without any overlap between the two groups (fig 1). There was no difference in acetaldehyde concentrations between those who were receiving anti-diabetic drugs and those who were treated with diet only. The decline of acetaldehyde concentrations in erythrocyte homogenates was exponential with two phases (fig 2). During the first phase no difference between the two groups was seen, whereas the flushers had a slower metabolism of acetaldehyde during the second phase (fig 3).

Discussion

The enzymatic nature of acetaldehyde metabolism in erythrocytes has been established by Inoue *et al.*¹⁵ The reaction is NAD-dependent and can be blocked by disulfiram, an inhibitor

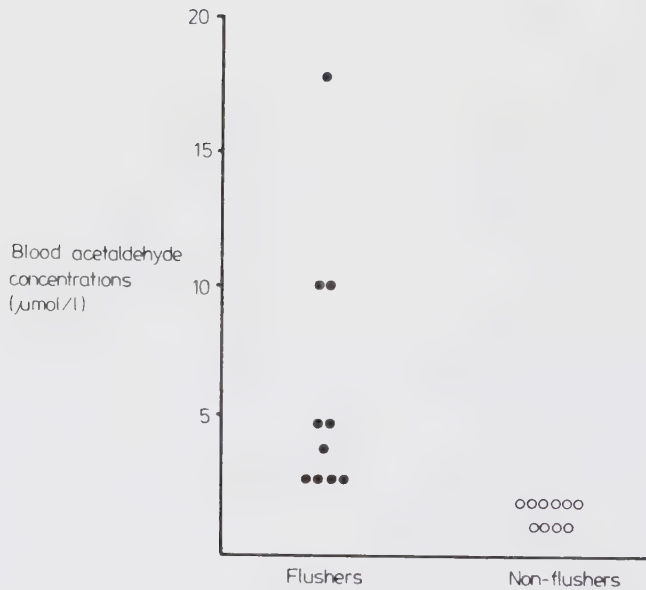


FIG 1—Increase of blood acetaldehyde concentrations during chlorpropamide-alcohol flushing challenge test.

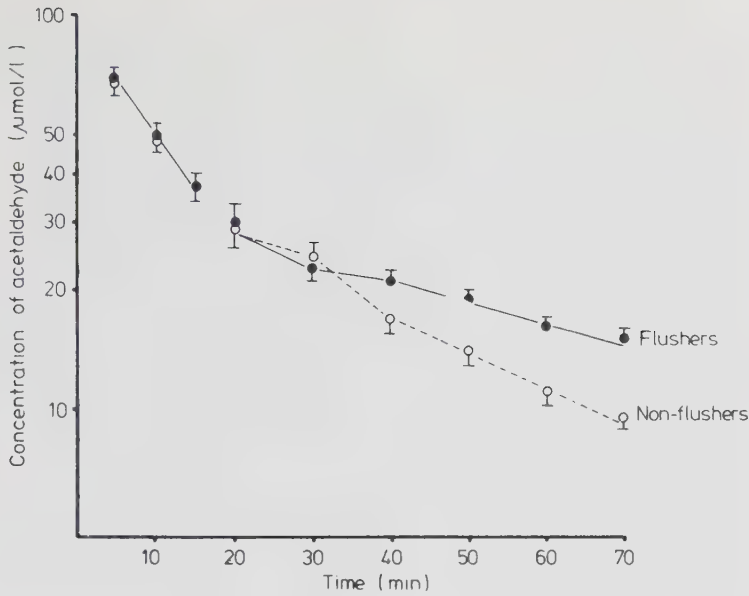


FIG 2—Elimination of acetaldehyde (initial concentration 100 $\mu\text{mol/l}$) in an erythrocyte homogenate with 1 mmol NAD/l. Two regression lines were calculated representing the two phases of acetaldehyde elimination, the first from 5 to 15 min and the second from 20 to 70 min. (Mean $\pm$ SEM.)

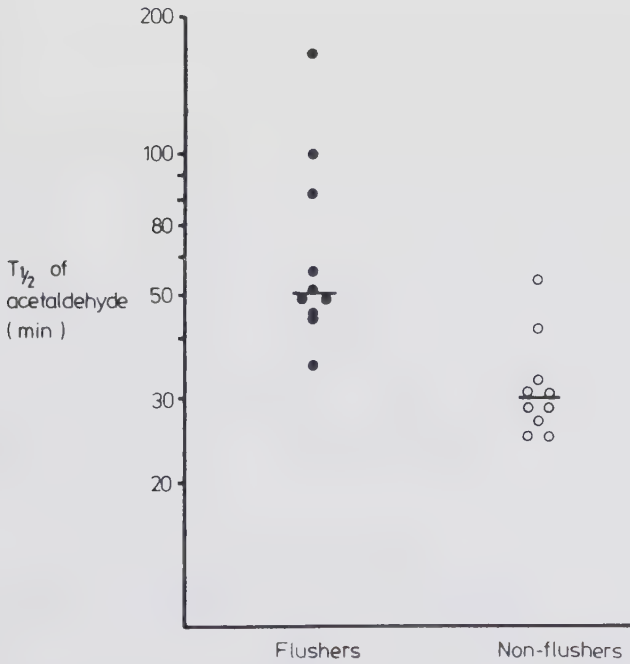


FIG 3—Individual half life ($T_{1/2}$) of acetaldehyde during the second phase (20-70 min) ($p < 0.005$).

of aldehyde dehydrogenase. Our finding of a biphasic acetaldehyde metabolism accords with earlier studies on purified sheep liver and human liver cytosolic enzyme.¹²⁻¹⁴ Biphasic kinetics may have several causes: (a) enzymes may exhibit co-operative behaviour,¹⁶ (b) different isoenzymes may be present, and (c) one enzyme may consist of non-equivalent subunits which have a different affinity for the substrate. The difference in metabolic rates between flushers and non-flushers was unlikely to be due to a difference in enzyme concentration, since the elimination in phase 1 was almost identical for the two groups. Our data support the hypothesis of a difference in aldehyde dehydrogenase activity between flushers and non-flushers, which is probably due to two types of high affinity binding sites for acetaldehyde. Hypothetically this difference in enzyme activity between those at risk of developing late complications and those who are not could be used after further refinement to identify patients at risk early by using a simple blood test. Determining the biochemical basis of protection against late complications through the change in enzyme activity will be an even more important step.

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ALDEHYDE DEHYDROGENASE ACTIVITY CORRELATES TO LATE DIABETIC COMPLICATIONS

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ABSTRACT

Type 2 diabetics, 26 with symptoms and/or signs of large-vessel disease (LVD-group) and 26 free from clinical vascular disease (FVD-group), were matched for sex, age, duration of diabetes after diagnosis and body weight.

The activity of erythrocyte aldehyde dehydrogenase (AldDH) was significantly higher ($p < 0.005$) in the LVD- than in the FVD-group, as indicated by a shorter half-life ($T_{1/2}$) of acetaldehyde in homogenates of erythrocytes and plasma (100.3 ± 11.1 min and 202.8 ± 28.2 min, respectively). The AldDH activity correlated to the prevalence of macroangiopathy and peripheral neuropathy ($p < 0.005$). This finding could not be explained by differences in therapy, blood glucose control, alcohol consumption or the presence of recognized risk factors of angiopathy. If differences in AldDH activity is primary or secondary to vascular diseases remains to be elucidated. If it is primary it may serve as a useful marker of increased risk of late complications. Manipulations of the activity, which is already known to be possible, may prove successful in delaying or preventing late diabetic complications.

Key words: Aldehyde dehydrogenase, chlorpropamide alcohol flush, diabetes mellitus, macroangiopathy, microangiopathy, peripheral neuropathy

Facial flushing after alcohol intake was reported as a side effect in diabetic patients on chlorpropamide already in 1956 (1), but only when Pyke's group 1979 and 1980 published papers on the relation between this chlorpropamide alcohol flush (CPAF) and risk of late diabetic complications it was realized that a new field in diabetology had been opened (2, 3). Soon several conflicting reports appeared, some supporting the original observations (4, 5), others casting doubt on the issue (6). The discrepancies could partly be explained by lack of objective methods to study the flush, and by differences between the populations studied (7).

The biochemical basis of the flush has been studied (8) in order to find a clue to the relationship between CPAF and the pathogenesis of vascular complications.

CPAF was found to be an antabuse-like reaction: chlorpropamide inhibits the aldehyde dehydrogenase (AldDH) leading to increased plasma acetaldehyde concentrations after alcohol intake (9, 10). This enzyme inhibition proved to be drug concentration dependent (11, 12). However, also differences in the basal erythrocyte AldDH activity between flushers and non-flushers were found (13), indicating the AldDH to be more sensitive to chlorpropamide-induced inhibition of ethanol metabolism in some diabetics. This finding of a difference in basal AldDH activity between CPAF positive and CPAF negative subjects prompted the present study.

METHODS

From our Diabetes Care Unit 150 type 2 diabetics, aged 50-70 years, and with a duration of diabetes of 3-10 years after diagnosis, were selected. The records of these patients were carefully examined. Forty-five patients with a history of angina pectoris, myocardial infarction, cerebral infarction and/or intermittent claudication and 45 patients without symptoms of macroangiopathy were selected. Patients with overt alcohol problem were excluded. After an interview and physical examination 26 pairs of patients, matched for sex, age, duration of diabetes and relative body weight,

remained. In no pair the patient of the group free from large-vessel disease (FVD-group) had more signs of retinopathy, nephropathy, or peripheral neuropathy than the patient of the group with large vessel-disease (LVD-group).

Seven of the 26 pairs were females. Less patients in the LVD-group were on a diet only regime than in the FVD-group (Table I). All patients on daily insulin treatment had been on sulphonylurea before the change of treatment 3-8 years earlier. No patient was on chlorpropamide. Insulin and sulphonylurea therapy was withheld for 24 hours before any part of the tests. The patients were told to refrain from alcohol, but were not fasting.

The probands were studied at two occasions, and those with positive CPAF test had an additional alcohol test without chlorpropamide premedication. First, they had a clinical examination done including family history, smoking habits, history of hypertension, ischemic heart disease, intermittent claudication and cerebral infarction and urine albumin excretion (Albustix) for the last three years. Foot pulses (dorsalis pedis and post-tibial pulses) were examined by palpation as well as an ultrasonic surface blood flow detector (Vasculascope). Vibration sense thresholds (Bio-thesiometer) were determined over the medial malleoli. The results were expressed in volts. Achilles tendon reflexes were examined with the patient kneeling on a chair. The retina was examined by ophthalmoscopy in mydriasis. Systolic and diastolic blood pressure were recorded indirectly in the right arm with a sphygmomanometer.

Two months later the patients had a single dose of 375 mg of chlorpropamide, (Diabines, Pfizer) in the evening 12 hours before the ingestion on the next morning of 8 g of pure ethanol in fruit juice. Skin temperature was recorded every five min at the lateral canthus of the left eye 20 min before and 30 min after the alcohol intake. The presence or absence of a facial flush was judged by the same examiner. Blood samples for acetaldehyde and chlorpropamide determinations were drawn before and 25-30 min after the alcohol ingestion.

After the last test all probands answered a questionnaire comprising 260 questions of which nine were alcohol related questions

(Mm-MAST). Possible alcohol over-consumption was considered when two or more "yes" answers were given (14).

Angiopathy and neuropathy scores

In order to compare the prevalence of complications to other variables measured scores were created. Since large-vessel disease was the criterion for selection of the probands this score was made the most important one.

Macroangiopathy score (0-12): A history of ischemic heart disease, cerebral infarction or intermittent claudication was 4 points each. However, without a history of claudication loss of foot pulses was given additional points: (0-4) corresponding to the number of non-detectable pulses.

Microangiopathy score (0-8): Retinopathy of simplex type was 3 points if severe, 2 moderate, and 1 if mild. Proliferative retinopathy was 4 points. Intermittent albuminuria was 1, persistent albuminuria was 2 if serum-Creatinine was less than 120 $\mu\text{mol/l}$, 3 if 120-200 $\mu\text{mol/l}$ or 4 points if more than 200 $\mu\text{mol/l}$.

Peripheral neuropathy score (0-4): Loss of Achilles tendon reflexes (A) and a threshold of vibration sense (V) of more than 40 units on the black scale of the Bio-Thesimeter was 4 points, 1A and >40V or 0A and 21-40V was 3, 2A and >40V or 1A and 21-40V or 0A and $\leq 20\text{V}$ was 2, and 2A and 21-40V was 1 point (4).

Complication score (0-24) was the sum of the three scores above.

Chemical analyzes

All analyzes were made without knowing whether the patient belonged to the LVD- or FVD-group. fS-Cholesterol, fS-Triglycerides, B-Glucose, Ery-HbA_{1c}, S-Creatinine were analyzed by hospital routine methods. Plasma chlorpropamide was determined by high pressure liquid chromatography (15). Blood acetaldehyde was determined by gas chromatography (9).

The activity of erythrocyte aldehyde dehydrogenase was assessed by the method of Inoue et al (16) modified as follows. At the first test occasion blood samples were collected from the patients in heparinized vacuum tubes, kept at 4°C, and within one hour centrifuged at 1000 x g at 4°C for 10 min. Plasma and erythrocytes

were mixed in fixed proportions (5:1) and homogenized by freezing (-70°C) and thawing. Three ml of the homogenate was incubated at 37°C with $100\mu\text{mol}$ acetaldehyde/l and 1 mmol nicotinamide-adenine dinucleotide (NAD)/l in 4-ml airtight glass vials. Samples were taken at fixed intervals and the reaction stopped by cooling the vials in iced water for 10 min followed by deproteinization with 0.5 ml 35% perchloric acid. After centrifugation at 4°C the clear supernatant was assayed for acetaldehyde using head-space gas chromatography (9). Diethylether served as internal standard in this part of the study. In our previous study we found that the decline of acetaldehyde concentrations in erythrocyte homogenates was exponential with two phases (13). During the first phase no difference between flushers and non-flushers was seen, whereas the flushers had a slower metabolism of acetaldehyde during the second phase (20-70 min). In the present experiments acetaldehyde metabolism was only studied during the second phase, from 30 to 60 min. The half-life ($T_{1/2}$) of acetaldehyde was calculated.

Statistics

The statistical significance of differences was assessed by both unpaired Student's t-test and Mann-Whitney U-test. For correlations, Spearman rank correlation coefficient was estimated. The results are given as mean $\pm$ SEM.

RESULTS

The patients of the LVD- and FVD-groups are presented in Table I. Presence of hypertension and hypertriglyceridemia were more common in the LVD- than in the FVD-group ($p < 0.05$). In Table II the complication scores are compared between the group with large vessel-disease (LVD) and the group free from vascular disease (FVD). The complication score was not significantly influenced by age or duration of diabetes in this study.

Aldehyde dehydrogenase activity

The AldDH activity during the incubation between 30 and 60 min was significantly higher in the LVD- than in the FVD-group: The acet-

Table I. A comparison between type 2 diabetics with (LVD-group) and free from (FVD-group) signs of large-vessel disease. Data for various factors are given as mean $\pm$ SEM or as number of individuals.

	LVD-group	FVD-group	p
N	26	26	
Sex (F:M)	7:19	7:19	
Age (years)	61.4 $\pm$ 1.1	61.8 $\pm$ 1.1	
Duration of DM (years)	5.7 $\pm$ 0.5	6.2 $\pm$ 0.5	
Therapy (diet only:			
sulphonylurea: insulin)	4:17:5	10:14:2	<0.05
First degree family history of DM	10	13	
Smoker	11	7	
Possible alcohol over-consumption	5	6	
Body weight (kg)	81.0 $\pm$ 3.5	77.3 $\pm$ 3.4	
Body weight of the ideal(%)	119 $\pm$ 4.0	113.8 $\pm$ 3.3	
Antihypertensive therapy	11	5	<0.05
Systolic blood pressure (mmHg)	155 $\pm$ 4	155 $\pm$ 3	
Diastolic blood pressure (mmHg)	91 $\pm$ 2	91 $\pm$ 2	
Fasting			
S-Cholesterol (3.8-8.3 mmol/l)	6.1 $\pm$ 0.2	5.7 $\pm$ 0.3	
S-Triglycerides (0.4-2.2 mmol/l)	2.5 $\pm$ 0.4	1.6 $\pm$ 0.1	<0.05
Random blood glucose (mmol/l)	12.6 $\pm$ 0.8	12.4 $\pm$ 0.7	
Erythrocyte HbA _{1c} (5.8-8.2 %)	10.4 $\pm$ 0.4	10.1 $\pm$ 0.3	

aldehyde elimination expressed as half-life ($T_{1/2}$) was 100.3 ± 11.1 min and 202.8 ± 28.2 min, respectively ($p < 0.005$) (Fig 1). Furthermore, the complication score correlated to the AldDH activity ($p < 0.005$) (Fig 2). A significant correlation was also found between AldDH activity and macroangiopathy score and neuropathy score, respectively ($p < 0.05$).

Diabetics treated with sulphonylurea or insulin had a higher AldDH activity than diabetics on diet only ($p < 0.005$). Females and males did not differ in the enzyme activity. The AldDH activity did not correlate to any recognized risk factor of vascular disease such as elevated serum lipids, hypertension, smoking, high alcohol consumption or poor diabetic control at the time of the test. Exclusion of patients with possible alcohol over-consumption did not change the results. The 13 patients of the FVD-group with high AldDH activity ($T_{1/2} < 150$ min) did not differ in the values of other factors from those 13 patients of the FVD-group with low AldDH activity ($T_{1/2} > 150$ min).

CPAF test

The result of the CPAF tests are presented in Table III. Two CPAF test positive probands also had a positive alcohol test and proved to be alcohol flushers. A true CPAF positive proband has increased acetaldehyde levels during the flush on CPAF test or, alternatively, does not show a placebo-alcohol flush (17). There were significantly more flushers in the FVD- than in the LVD-group ($p < 0.05$). However, if only true CPAF reactions were considered the difference did not reach statistical significance.

Blood acetaldehyde levels during CPAF tests compared to basal AldDH activity

Subjects with chlorpropamide levels outside one SD from the mean were excluded since the drug-induced inhibition of AldDH is concentration dependent (11). The quartile of diabetics with the lowest AldDH activity had significantly higher acetaldehyde levels than the quartile of patients with the highest enzyme activity: 1.4 ± 0.3 $\mu\text{mol/l}$ ($N=11$) and 0.6 ± 0.1 $\mu\text{mol/l}$ ($N=12$), respectively ($p < 0.05$).

Table II. Angiopathy and neuropathy in one group of type 2 diabetics with signs of large vessel-disease (LVD-group) and one group free from vascular disease (FVD-group). Data are given as mean $\pm$ SEM.

	LVD-group (N=26)	FVD-group (N=26)	p
History of			
Ischemic heart disease	19	0	<0.001
Intermittent claudication	7	0	<0.01
Cerebral infarction	3	0	
Palpable foot pulses	2.0 $\pm$ 0.3	3.8 $\pm$ 0.1	<0.001
Doppler positive foot pulses	3.3 $\pm$ 0.2	4.0 $\pm$ 0	<0.005
Macroangiopathy score (0-12)	5.5 $\pm$ 0.4	0 $\pm$ 0	<0.001
Retinopathy score (0-4)	0.4 $\pm$ 0.2	0 $\pm$ 0	
Albuminuria score (0-4)	0.3 $\pm$ 0.1	0 $\pm$ 0	
S-Creatinine (60-115 μ mol/l)	97 $\pm$ 4	99 $\pm$ 3	
Microangiopathy score (0-8)	0.8 $\pm$ 0.2	0.1 $\pm$ 0.1	<0.01
Remaining achilles tendon reflexes	1.2 $\pm$ 0.2	1.8 $\pm$ 0.1	<0.01
Vibration threshold (units)	30 $\pm$ 2	23 $\pm$ 2	<0.05
Peripheral neuropathy score (0-4)	1.8 $\pm$ 0.2	0.8 $\pm$ 0.2	<0.005
Complication score (0-24)	8.0 $\pm$ 0.5	0.9 $\pm$ 0.2	<0.001

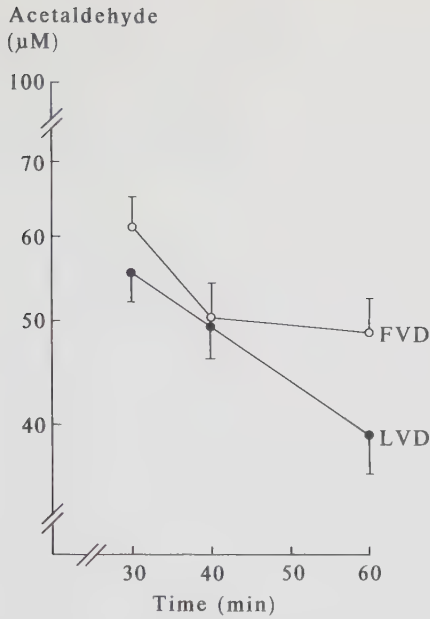


Fig.1. Elimination of acetaldehyde (initial concentration of 100 μM) in an erythrocyte homogenate with 1 mmol NAD/l. The elimination curves from 30 to 60 min were calculated for the two groups of type 2 diabetics; with signs of large-vessel disease ($\bullet$) and free from clinical vessel disease ($\circ$), respectively (mean and SEM).

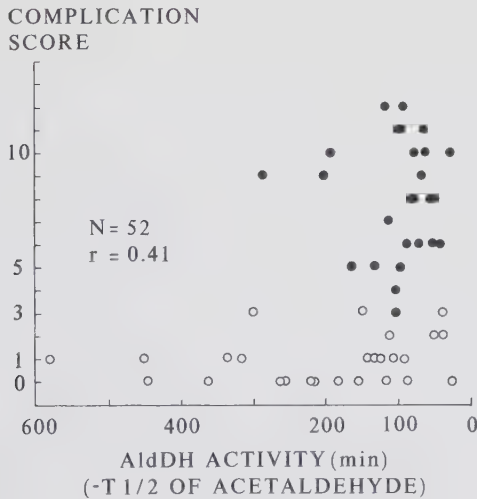


Fig.2. Aldehyde dehydrogenase activity (= - half-life of acetaldehyde 30-60 min) compared to a complication score in 26 type 2 diabetics with signs of large-vessel disease ($\bullet$) and 26 free from clinical vessel disease ($\circ$).

Table III. CPAF tests in 45 type 2 diabetics.

Sex distribution, skin temperature, plasma chlorpropamide and blood acetaldehyde levels, and diabetic complications are compared between true CPAF-positive and CPAF-negative subjects. Data are given as mean $\pm$ SEM.

	True CPAF (N=13)	CPAF-negative (N=32)	p
Sex (F:M)	8 : 5	6 : 26	<0.01
Basal skin temperature ($^{\circ}$ C)	31.3 $\pm$ 0.4	32.1 $\pm$ 0.3	
Increase in skin temperature ($^{\circ}$ C)	1.2 $\pm$ 0.1	0.6 $\pm$ 0	<0.001
Plasma chlorpropamide (mg/l)	36.7 $\pm$ 2.3	32.1 $\pm$ 1.5	<0.05
Blood acetaldehyde (μ mol/l)	2.5 $\pm$ 0.3	0.8 $\pm$ 0.1	<0.001
Large vessel disease group (N=23)	4 (17%)	19 (83%)	
Free from vascular disease group (N=22)	9 (41%)	13 (59%)	

DISCUSSION

The results of the CPAF tests confirm previous results: plasma chlorpropamide concentrations were higher in flushers than in non-flushers, blood acetaldehyde levels were more markedly elevated in flushers and diabetics with a low AldDH activity than in non-flushers and diabetics with a high AldDH activity (9, 11, 13).

A high aldehyde dehydrogenase (AldDH) activity was found in diabetics with late complications (LVD-group), especially large vessel-disease, compared to diabetics free from clinical vascular disease (FVD-group). This difference was more marked than differences in prevalence of hypertension, hyperlipidemia, CPAF, smoking and blood glucose control. This study was not designed to investigate a relation between microangiopathy and AldDH activity. However, studies are in progress. It is important to point out that the AldDH activity determinations were made without knowing other results of the study.

Is the difference in AldDH activity a primary or secondary phenomenon? Which role does AldDH play in the pathogenesis of vascular disease? Presently, these questions could not be answered. However, there are some arguments in favour of the AldDH activity being a primary phenomenon.

1. Differences in hepatic cytosolic AldDH activity has been suggested to be a primary abnormality predisposing to alcoholism (18). Erythrocyte AldDH shares several characteristics with the hepatic cytosolic enzyme. The biphasic nature of kinetics suggests both high and low affinity sites for acetaldehyde (19).

2. CPAF has been shown to be an inherited trait in some type 2 diabetics (20). CPAF was significantly more common and blood acetaldehyde levels were higher in diabetics with low enzyme activity (13).

3. In about 50% of Japanese there is one AldDH isoenzyme lacking, causing high concentrations of acetaldehyde in blood and alcohol flushing (21). Diabetics in Japan have been reported to be less prone to develop macrovascular complications than Caucasians (22).

4. There was no association between the finding of reduced AldDH activity and abnormalities of routine serum liver function test (23).

5. Both low and high AldDH activity were found in the group clinically free from vascular disease, while nearly all in the LVD-group had high enzyme activity. However, only a prospective study could show if those in the FVD-group with high AldDH activity later will develop signs of angiopathy.

6. Alcohol consumption depresses hepatic AldDH activity, while a reduction in the alcohol intake is followed by a rise in the enzyme activity (24).

In this study the influence of alcohol consumption was the same in the two groups. Interestingly, a small daily alcohol intake, which would reduce the AldDH activity, has been shown to protect against future vascular complications in non-diabetics (25, 26). Chlorpropamide might also depress erythrocyte AldDH activity, however, medication with other sulphonylurea drugs was not associated with low enzyme activity in this study.

Whether the reported difference in aldehyde dehydrogenase activity between type 2 diabetics with late complications and those free of manifest angiopathy is an important factor involved in the pathogenesis of angiopathy or not remains to be proved.

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